BEHAVIORAL, DEVELOPMENTAL, AND EVOLUTIONARY MECHANISMS OF SOCIALLY GUIDED VOCAL LEARNING OF BIRDSONG

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Songbirds are the most common research model of human speech development, due to parallels in their behavioral, developmental, and neural mechanisms of learning. However, similarities in social influences on vocal learning remain largely unknown. Human infants utilize socially guided vocal learning, the ability to use social feedback contingent on immature vocalizations to guide vocal development. This is thought to be a rare and unusual capacity, and has only been previously demonstrated in one species of songbird. This dissertation proposes that socially guided vocal learning is more common than previously supposed, and describes the first causal evidence of this learning strategy in the zebra finch (*Taeniopygia guttata*), the most ubiquitous laboratory songbird. I explore the developmental and evolutionary prerequisites required for socially guided vocal learning to emerge, as well as the necessary neural connectivity between social motivation and vocal learning brain regions. I present findings from an experiment investigating how non-vocal feedback from zebra finch females over a video display affects vocal learning in juvenile males. Males which viewed a female arousal behavior presented contingently on their song production learned song with greater fidelity than yoked controls. I then use a longitudinal study to demonstrate that zebra

finch parents respond contingently to the songs of their sons in a naturalistic family context, and that the timing and frequency of this feedback predicts song learning outcomes. Additionally, I investigate the neuroendocrine mechanisms of socially guided vocal learning by testing the hypothesis that the nonapeptide hormone arginine vasotocin (AVT, the avian homologue of vasopressin) plays an organizational role in species-typical development of social and affiliative behaviors, and resulting song learning outcomes. Finally, I expand my findings beyond zebra finches with an evolutionary model of how particular traits grant certain passerine species the developmental opportunity and functional impetus necessary to evolve socially guided vocal learning, which proposes uninvestigated species in which socially guided vocal learning may exist. Overall, my research presents evidence for a previously unknown, socially guided vocal learning strategy in the zebra finch, explores its underlying mechanisms, and emphasizes the importance of studying communicative systems within a social context.

BIOGRAPHICAL SKETCH

As the daughter of a diplomat, Samantha Carouso grew up in the United States, Greece, the Dominican Republic, South Africa, Australia, Thailand, Cyprus, and Indonesia. Her interest in avian behavior began with a childhood fascination with penguins, which famously resulted in a black-footed penguin stealing her shoe and waddling off with it into the Indian Ocean. She graduated class salutatorian from the International Baccalaureate program at the International School of Bangkok in 2006, completing her thesis on problem solving in Thai large-billed crows. She received a B.A. in Biology, specializing in Animal Behavior, from Williams College in 2010, during which time she spent her summers at AKTI Environmental NGO studying endemic species in the demilitarized zone in Cyprus, and researching vocal learning in African grey parrots in the lab of Dr. Irene Pepperberg at Brandeis University. After graduating, she spent two years at the Cox Lab of Visual Neuroscience at the Rowland Institute at Harvard, conducting electrophysiological research on the visual object recognition system in rats.

In September 2012 she entered the Psychology graduate program at Cornell University, and joined the developmental psychobiology lab of Dr. Michael Goldstein. Her doctoral research, supported by a National Science Foundation Graduate Research Fellowship, focused on vocal learning in the zebra finch, particularly the role of social feedback from conspecifics in guiding the songs of juveniles towards more mature forms. Her work has been published in *Current Biology*, *Proceedings of the Royal Society B,* and *Scientific American*, and featured in *Science.* During her time at Cornell

she also developed a passion for teaching, and taught two original psychology seminar classes (Animal Cognition and Comparative Development), mentored over thirty undergraduate research assistants and eleven honors theses, created psychology exhibits for the Ithaca Sciencenter, participated annually in the Expanding Your Horizons science workshops for middle school girls, and won a Russell Distinguished Teaching Award. She married parasitologist David Thomas Peck, Ph.D, at the Cayuga Nature Center in 2016. She and David will begin post-doctoral positions with the Cornell Active Learning Initiative this summer.

To my parents, who encouraged me relentlessly and let me bring home injured birds

And to David, my mutual biggest fan

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CHAPTER 1

INTRODUCTION: A BIRD'S-EYE VIEW ON THE DEVELOPMENT OF COMMUNICATION

Since the original pioneering work of Peter Marler on the vocal ontogeny of sparrows (Marler, 1970a), the learning of birdsong has captivated scientists as a study system, due to its behavioral, neural, and developmental parallels with speech development in human children (Marler, 1970b; Kuhl, 2003). Since the time of Marler's sparrow studies, the world's most popular study species has shifted to the zebra finch (*Taeniopygia guttata*), due to its rapid development, simple song, and ease of breeding in captivity (Zann, 1996). The mechanisms of song learning found in sparrows were used as foundational principles to guide investigations into vocal ontogeny in all other songbird species. However, sparrows are somewhat unusual among songbirds, in that adult males are highly territorial and aggressively exclude other males from their territory (Marler & Peters, 1982). This presents a challenge for young sparrows, which must learn song by eavesdropping on adults from afar, and are unable to interact directly with their song tutors (Beecher et al., 2007). It was therefore assumed that all songbirds could learn via eavesdropping, and juveniles did not require the social interaction and feedback found to be necessary for normal speech development in human children (e.g. Goldstein, King & West, 2003). Even on the occasions when scientists have looked for evidence of social influences on song learning, they often found nothing (e.g. Houx & ten Cate, 1998). When such feedback behaviors were first discovered, by video-taping brown-headed cowbird females

(West & King, 1988), it became clear why they were easy to overlook: they were too rapid for the human eye to detect without slowing the tape down.

Imagine an alien species with a much slower perception of time than humans. An acorn falling from a tree would appear to teleport from the branch to the ground with no descent in between. For the aliens, perceiving human actions would be like watching a time-lapse movie in which motion appears to be sped up. Watching a gymnastics floor routine would be baffling – the alien would be unable to perceive the rapid movements the gymnast is executing – all it would see is a person suddenly moving from one end of the mat to the other. Why, it wonders, are the human judges so excited by someone moving across the room? This is often the way humans perceive the movements of small animals that have a more rapid perception of time. Animals often experience the world in ways that are beyond human capacities, therefore if we don't take their perceptual systems into account we may overlook sources of social information for that species. Solving the mysteries of animal behavior often involves immersing ourselves in their perceptual world, taking a literal bird's eye view of their behaviors.

Striking recent examples from birds were only discovered when they were filmed with high-speed cameras, allowing us to view their behaviors in 'bird time', the far finer temporal scale in which birds are capable of viewing the world. The manakins are a family of birds best known for elaborate, ricocheting, moonwalking courtship displays, with the exception of the black manakin. Its display – we thought – consists only of rapid, small hops. Yet when filmed and played slowed down, researchers discovered that each hop was in fact a 360-degree backwards somersault, lasting 0.37

seconds, and completely imperceptible to the human eye (Lindsay et al., 2015). Similarly, the cordon bleu finch, a common pet bird, was thought to have a simple hopping courtship display. When slowed down, they were revealed to be 'tap-dancing', alternating from foot to foot (Ota, Gahr & Soma, 2015). In the case of both the manakin and the finch, although their displays initially appear simple and uninteresting to humans, females of the species pay close attention to male displays. They are very picky about which they prefer, as these rapid displays are challenging to execute correctly and provide a wealth of information about the physical quality of the male.

These ideas are still novel to many scientists. To study animals appropriately, we need to study them through the lens of their own *umwelt*, the way in which a particular organism experiences the world. How animals' perceptual capacities allow them to see their environment, how evolution has shaped them to utilize different sources of information, and how their social lives are structured all influence the ways in which they learn and think. This idea is critically important for the study of communication.

What would happen if we applied this idea of 'bird time' to song learning in the zebra finch? Song learning in these birds has been studied for decades, but almost never in a social context. Usually, researchers let birds expose themselves to adult song by pecking a key, and their subsequent learning is assessed (Tchernichovski et al., 1999). Based on these studies, zebra finches were thought to learn solely via imitation, like sparrows, with no social information required. What information might exist in the immediate environment that a young learner could utilize? There has been

far more research done on male singing behavior than on the female reactions to song. Since females are the arbiters of successful song – they judge whether a male's song is good enough to select him as a mate – understanding the evolution and development of communication requires study of young learners and female listeners as a linked system of mutual influences.

As adult human researchers studying birds, we are aliens attempting to decipher a foreign way of perceiving the world. To discover the behaviors we cannot see ourselves, we need to pay attention to the reactions of the intended audience, in this case female birds responding to male displays. My research aims to elucidate social influences on vocal development in the zebra finch from the perspective of senders and receivers, and on multiple levels of analysis, with the assumption that learning processes and social behaviors have multiple causes, and may operate simultaneously on multiple such levels (Tinbergen, 1963). This dissertation investigates the mechanisms underlying socially guided vocal learning of bird song from behavioral, developmental, neuroendocrine, and evolutionary perspectives.

In Chapter 2, I discuss the history of songbird studies, insights from considering 'bird time' when studying animal social behaviors, and the startling discovery of socially guided vocal learning in the cowbird. I link these ideas to song development in the zebra finch, the numerous studies which have hinted at social feedback from non-singing females playing a role in their vocal development, and proposed neural mechanisms by which social motivation brain circuitry and vocal learning brain circuitry may be connected. In Chapter 3, I present findings from the first ever study causally demonstrating that vocal learning is influenced by non-vocal

feedback behaviors displayed by female zebra finches contingent on the immature song of juvenile males. I showed young males videos of females performing 'fluff-ups', an arousal behavior (Vyas et al., 2009), in response to their own production of immature song. Males which received this contignent social feedback while they were learning ultimately produced better song. In Chapter 4, I present data from a longitudinal study examining the timing and prevalence of, and impact on vocal learning of, contingent feedback behaviors from parents in a naturalistic family environment. Mothers and fathers were found to selectively respond to their songs of their sons with fluff-ups and contingent song, respectively, which predicted juvenile song learning outcomes. In Chapter 5, I present results from two experiments investigating the organizational role of a nonapeptide hormone – arginine vasotocin (AVT), the avian homolog of vasopressin - on zebra finch social behaviors, and its impact on song learning outcomes. Chicks receiving intracranial injections of AVT in the first week after hatching were more socially motivated later in life and learned song more accurately than saline controls, while those injected with a vasotocin antagonist showed the opposite results. In Chapter 6, using data from 28 different species of passerine, I present an evolutionary model of developmental and life history traits predicting the presence or absence of a socially guided vocal learning strategy in a given species. Gregarious species which use song for attracting a mate rather than defending territory, and have a developmental overlap in the sensitive periods during which song is memorized and practiced, are predicted to be more likely to utilize social feedback to guide song learning.

REFERENCES

Beecher, M. D., Burt, J. M., O'Loghlen, A. L., Templeton, C. N., & Campbell, S. E. (2007). Bird song learning in an eavesdropping context. *Animal Behaviour, 73,* 929-935.

Goldstein, M. H., King, A. P., & West, M. J. (2003). Social interaction shapes babbling: testing parallels between birdsong and speech. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(13), 8030–8035.

Houx, B.B., & ten Cate, C. (1998). Do contingencies with tutor behaviour influence song learning in zebra finches? *Behaviour, 135*(5), 599-614.

Kuhl, P. (2003). Human speech and birdsong: Communication and the social brain. *Proceedings of the National Academy of Science, 100*(17), 9645-9646.

Lindsay, W.R., Houck, J.T., Giuliano, C.E., & Day, L.B. (2015). Acrobatic courtship display coevolves with brain size in manakins (Pipridae). *Brain, Behavior, and Evolution, 85,* 29-36.

Marler, P. (1970a). A comparative approach to vocal learning: Song development in white-crowned sparrows. *Journal of Comparative and Physiological Psychology, 71,* 1-25.

Marler, P. (1970b). Birdsong and speech development: could there be parallels? *American Scientist, 58,* 669-73.

Marler, P., & Peters, S. (1982). Developmental overproduction and selective attrition: new processes in the epigenesis of birdsong. *Developmental Psychobiology, 15,* 369-78.

Ota, N., Gahr, M., & Soma, M. (2015). Tap dancing birds: the multimodal mutual courtship display of males and females in a socially monogamous songbird. *Scientific Reports, 5,* 16614.

Tchernichovski, O., Lints, T., Mitra, P.P., & Nottebohm, F. (1999). Vocal imitation in zebra finches is inversely related to model abundance. *Proceedings of the National Academy of Science, 96,* 12901-12904.

Tinbergen, N. (1963). On aims and methods of Ethology. *Zeitschrift Für Tierpsychologie, 20*(4), 410–433.

Vyas, A., Harding, C., Borg, L., & Bogdan, D. (2009). Acoustic characteristics, early experience, and endocrine status interact to modulate female zebra finches' behavioral responses to songs. *Hormones and Behavior, 55*, 50-59.

West, M.J., & King, A.P. (1988). Female displays affect the development of male song in the cowbird. *Nature, 334*, 244-246.

Zann, R.A. (1996). *The Zebra Finch: A Synthesis of Field and Laboratory Studies* (Oxford University Press, Oxford).

CHAPTER 2

LINKING VOCAL LEARNING TO SOCIAL REWARD: PROPOSED NEURAL MECHANISMS OF SOCIALLY GUIDED VOCAL LEARNING

Abstract

The social environment plays an important role in vocal development. In songbirds, social interactions that promote vocal learning are often characterized by contingent responses of adults to early, immature vocalizations. Parallel processes have been discovered in the early speech development of human infants. Why does contingent social feedback facilitate vocal learning so effectively? Answers may be found by connecting the neural mechanisms of vocal learning and control with those involved in processing social reward. Here we extend the idea of Newman's social behavior network, a tightly interconnected system of limbic areas across which social behavior and motivation are distributed, to an avian social/vocal control network. We explore anatomical and functional overlaps between song circuitry and social-motivational circuitry. We find that circuitry linking basal ganglia with cortical areas serves to integrate social reward with vocal control and may underlie socially guided vocal learning. In species that have evolved socially guided vocal learning, a unique link has been forged between social circuitry and vocal learning systems, such that learning is driven by social motivation.

Introduction

Vocal learning, the ability to modify vocalizations as a result of experience with other individuals, is a rare phenomenon. Although the evolutionary lineage leading to humans diverged from that leading to songbirds 300 million years ago, the process by which birds learn to sing and humans learn to speak share parallels at multiple levels. Humans and songbirds must both achieve the complex task of learning to produce sounds which are functional for communicating with conspecifics. Song and language both require learning during a critical developmental period, and practice through immature vocalizations for both birds (subsong and plastic song) and babies (babbling).

An additional, and understudied, parallel is the powerful role of social feedback in the development of mature vocal forms. Evidence is rapidly accumulating that vocal learning in humans and songbirds is motivated by social factors and is intrinsically rewarding at the neural level. Functional and neural links between social-motivational brain regions and vocal learning circuitry continue to emerge from new investigations. Without social exposure, both humans and songbirds fail to develop normal vocalizations. Immature vocalizations play an essential role, not only in learning to use the vocal apparatus, but also in eliciting feedback from social partners to guide immature vocalizations into more mature forms. This chapter will assess mechanisms of vocal learning with respect to the ecological contexts of young learners. A crucially important context, especially in altricial species, is the social environment.

Early work on vocal development across species found that, for both bird song

and human language, learning primarily requires exposure to species-typical sounds during a sensitive period. Experimental manipulations found the amount of input necessary to be small, and effective regardless of the inclusion of social factors, provided that the learning organism had extensive time to practice (i.e. Lenneberg, 1967; Marler, 1970). While this paradigm led to increased understanding of the neurological control of vocal production, researchers investigating the ontogeny of communication began to note that it could not explain all that they observed. Social stimulation, or lack thereof, can extend or delay the sensitive period for song learning in birds, or even allow vocalizations to be modified throughout life (Baptista & Gaunt, 1997; Payne & Payne, 1997). Different vocalizations may be utilized in different social contexts, and vocal learning does not merely involve learning to produce sounds, but also when and how to use them appropriately. If raised in an inadequate social environment, cowbirds may develop potent songs but not know how to use them (West et al., 1990), vervet monkeys may learn alarm calls but use them in response to non-threatening stimuli (Seyfarth & Cheney, 1986), and marmosets may learn vocalizations but fail to learn to take turns when communicating with conspecifics (Takahashi et al., 2016). While parrots may learn to mimic human speech through mere exposure, they can only learn to use language referentially and functionally when taught using socially interactive techniques (Pepperberg, 1993). Social partners may influence vocal development through a variety of mechanisms, providing learners with reinforcement, an attentional focus, general stimulation, or selective feedback.

Not all vocally learning species are equally socially influenced, necessitating a

comparative, cross-species approach to understand what traits grant a given species the greatest capacity for vocal flexibility during ontogeny. Species with the most unpredictable environments, such as the zebra finches of central Australia, and the greatest mobility, such as migratory birds and mammals, tend to have the greatest capacity for learning new vocalizations and being influenced by social factors. This may be due to selective evolutionary pressures placed on species which would be most likely to encounter unfamiliar conspecifics with different vocal dialects. Species which live in stable, consistent social groups year-round would gain less advantage from vocal plasticity, and are often less flexible vocal learners (Snowdon & Hausberger, 1997). The developmental mechanisms underlying the incorporation of social information into learned vocalizations also vary depending on a given species' ontogeny, sensitive periods, life history, social structure, access to vocal tutors early in life and, crucially, usage of vocalizations. Bird species which use song primarily for defending territories from competitors, and therefore benefit most by learning songs directly from dominant males, should be expected to learn song very differently from those who use song only for attracting a mate, and may benefit most from paying attention to which songs are most arousing to the opposite sex.

In the study of bird song development, two primary models of learning processes have been proposed: instructive and selective (Changeux et al., 1984; Jerne, 1967). Instructive models propose that stimulation from the environment adds information not already present in the behavioral repertoire. Instructive models typically consist of young birds listening to a tutor's song, memorizing it, and subsequently practicing until they can reproduce the song (e.g. sensorimotor learning;

Konishi, 1965). Selective models propose that learning consists of experience leading to the selection and attrition of behaviors from a relatively vast pre-existing repertoire. The best known example of selective learning is "action-based learning" (Marler, 1991), also called "selective attrition" (Marler & Peters, 1982). Primarily studied in territorial sparrows, action-based learning refers to the selection of songs from a large, overproduced repertoire sung during the plastic stage of song learning. When territorial male sparrows engage in counter-singing, they exchange similar song types. During these social interactions, matching songs may be reinforced, while non-matching types are discarded (Marler & Nelson, 1993). Young song sparrows are more likely to select matching songs from tutors they can overhear interacting with other birds than from those with which they can directly interact, and do not learn preferentially from more aggressive or higher-quality adults (Akçay et al., 2014; Beecher, 2016). While both instructive and selective models explain numerous aspects of song learning, especially the eavesdropping-based (Beecher et al., 2007) song learning strategy in territorial sparrows, both models rely heavily on imitation. Neither explains invention and improvisation of new song types which vary from that of the tutor, or the learning process of any species which utilizes non-vocal feedback or otherwise develops without exposure to an auditory model. The socially guided learning (SGL) model instead proposes that social partners may selectively reinforce components of immature vocalizations. Much like action-based learning, SGL relies on behavioral shaping, allowing an animal to retain those behaviors most often associated with a positive social response, but rather than relying on selective attrition of non-functional songs, SGL allows young learners to construct mature vocalizations

from component sounds. When attempting to write an essay, we find it far easier to be given a blank page and construct the essay using our vocabulary rather than being given a list of all possible combinations of all possible words and whittling it down to only those words we wish to include. In the same way, it is easier for a developing organism to construct an adaptive vocalization from basic parts than by being born already able to produce all possible vocalizations and removing those elements which are non-functional. While action-based learning incorporates aspects of SGL, it only allows for social shaping through selective attrition, not the constructive mechanisms we propose.

Socially Guided Learning in Birdsong Function and Development

There exist over 4000 species of songbird (oscine), and no two are precisely alike in ecological niche, life history strategy, or song learning trajectory. The degree of social interaction necessary and sufficient for normal vocal development varies across species. Song serves two primary functions in birds: to declare a territory from which other birds are aggressively excluded, and to attract members of the opposite sex for mating (Catchpole & Slater, 1995; Kroodsma & Miller, 1996), though some species employ only one of these song functions. In many species of songbird only males sing, though there are numerous species in which females also produce song (Odom et al., 2014). There is extreme diversity in the types of songs birds produce, and each individual species has a characteristic acoustic structure. The simplest unit of the song is referred to as an ‘element’ or ‘note’. A series of elements that regularly occur together from a song ‘syllable’, while a sequence of multiple syllables that

repeatedly occurs in a song is described as a 'motif' (Brenowitz et al., 1997) (see Figure 2.1). Most juvenile songbirds fail to develop normal song if they do not hear the song of a conspecific adult tutor, or if they cannot hear themselves sing.

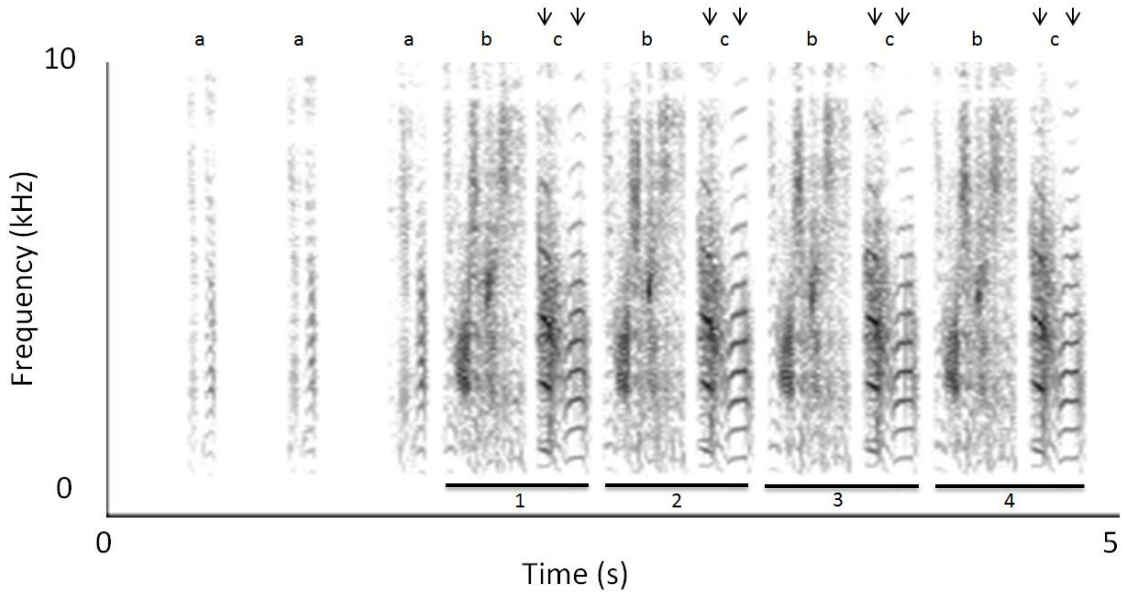


Figure 2.1. **Spectrogram of adult zebra finch song with labeled structural components**. The song begins with repeated introductory notes ('a') followed by a motif which is repeated several times (bars 1-4). Motifs consist of a number of syllables (identified by letters above the spectrogram). Syllables may contain one or more elements or notes. For example, syllable 'c', which is repeated four times, consists of two notes (denoted by arrows).

Songbirds may be divided into 'open-ended' and 'close-ended' or 'age-limited' learners (Nottebohm, 1993). Open-ended learners, including canaries (*Serinus canarius*), red-winged blackbirds (*Agelaius phoenceus*), and European starlings (*Sturnus vulgaris*), can continue to learn new songs or song elements for many years or throughout life (Adret-Hausberger et al., 1990; Yasukawa et al., 1980). For close-ended learners, song acquisition is restricted to a short sensitive phase, usually early in development. Research on vocal learning in birds has been guided by the sensorimotor model, based on studies of song learning in the white-crowned sparrow (*Zonotrichia*

leucophrys), a close-ended learner (Konishi, 1965). This model incorporates two developmental stages: the sensory period, during which the song is acquired and memorized, and the sensorimotor period, during which the bird practices the song and uses auditory feedback to compare its own song to its stored memory. The beginning of the sensorimotor phase is accompanied by the production of *subsong*, the first song-like vocalizations, but which are unstructured, vary from moment to moment, and bear little resemblance to adult song. Its variability invites comparison with the early stages of babbling in human infants. Subsong and baby babbling both serve to train the vocal apparatus and improve vocal control, as well as to elicit social feedback to facilitate development of more mature sounds (Goldstein et al., 2003). Subsong gradually develops into *plastic song* that incorporates recognizable syllables from the song model, but remains variable and requires additional practice before it will mature into the final, *crystallized* adult song (DeWolfe et al., 1989). Syllable structure tends to reach an adult form prior to the onset of crystallized syntax, such that even after learning to produce mature and stereotyped song elements, young birds will still rearrange the sequence of these elements between song bouts. The crystallization process is rapid compared to the prolonged learning period preceding it (Todt & Geberzahn, 2003). The duration of the sensitive period is not fixed, but may vary depending on social experience. For many species, raising birds in isolation extends the sensitive period, such that adults may still learn song elements when a tutor is finally presented (Slater et al., 1988). For some species, birds exposed only to the song of a different species during development will continue to learn songs from conspecifics at a time when normally-raised birds can no longer learn new song (Slater

et al., 1988). Insufficient social experience or exposure to the tutor leaves the brain open to learning for longer than normal.

The subject of sensitive periods in the development of song has led to some debate on the differing effects of tutoring birds using live, interactive social partners versus pre-recorded tapes of birdsong. There are a few oscine species in which naïve individuals may produce near-perfect copies of tape-recorded song, including chaffinches (Thorpe, 1958) and white-crowned sparrows (Marler, 1970), which were among the first and most commonly studied model species. Under natural conditions, these species learn via eavesdropping on neighboring adult males while establishing territories (Beecher et al., 1994; Nice, 1943). It is important to note, however, that social influences can dramatically change song learning, and white-crowned sparrows still learn more readily from a live tutor than a recording (Baptista & Petrinovich, 1984). Early tape-tutoring isolate studies concluded that white-crowned sparrows uniformly reject heterospecific song (Marler, 1970), but when the tutor is a live bird they will learn from another species (Baptista & Petrinovich, 1984). Furthermore, while conspecific tape-tutored songs are deemed 'normal' to the ears of researchers, they are often functionally useless. A study of tape-tutored wood thrushes (*Hylocichla mustelinu*) concluded that they developed normal wild-type song, but when the song was played back to wild wood thrushes they failed to elicit any response (Lanyon, 1979). Many other species fail to learn normal song entirely when solely exposed to tape recordings (Baptista & Petrinovich, 1986; Deregnaucourt, et al., 2013; Thielcke, 1970). Facultative social learners can use recordings to form a song model memory in isolation, but their learning is greatly improved with exposure to a live tutor. Indigo

buntings (*Passerina cyanea*), domestic canaries, and European starlings can all learn a few syllables from a recording, but learn far more when exposed to the same song produced by a live tutor (Chaiken et al., 1993; Rice & Thompson, 1968; Waser & Marler, 1977). Obligate social learners, such as Eurasian tree-creepers (*Cethia familiaris*) and North American sedge wrens (*Cistothorus platensis*)[1], do not learn from tape recordings, but will readily learn from one another when naïve individuals are housed together (Kroodsma & Verner, 1978; Thielcke, 1984). Human infants seem to be subject to similar learning constraints, as studies of children raised in isolation found that they fail to develop speech normally (Fromkin et al., 1974; Lane, 1976). It is important to remember, however, that when a social organism such as a human or songbird is raised in isolation it is deprived not only of normal exposure to vocalizations but also of all typical social exposure. As in the case of isolate-reared monkeys developing severe behavioral abnormalities (Harlow & Harlow, 1962), early social deprivation likely has dramatic developmental impacts beyond vocal learning.

The impact of social factors also seems to shift over the course of development. For example, white-crowned sparrows will readily learn from a tape recording until 50 days of age, but will only accept live tutors as song models past that point (Baptista & Petrinovich, 1986). Conversely, starlings learn better from live tutors than tapes at 4 months of age, but tape tutoring becomes more effective by 12 months (Chaiken et al. 1993). A possible reason for this variation may be the difference in the repertoire sizes of these two species. White-crowned sparrows rarely sing more than one song as adults (Baptista, 1975), while starlings can sing dozens of

[1]When housed in acoustic isolation or exposed to passive playback, sedge wrens will improvise song elements, resulting in an approximation of species-typical song.

different song types (Van Hout et al., 2012). This may impose different constraints on learning, such that it becomes too restrictive for a species with a large repertoire to limit learning to only one familiar tutor.

Socially Guided Vocal Learning in the Zebra Finch

Each oscine species has its own learning requirements and capabilities, and no single species can serve as a model of vocal learning for all oscines. However, the species which has been most thoroughly studied and whose learning mechanisms have been most often compared to those of humans is the zebra finch. For this species, live social interaction of the correct form and timing is vital for normal song learning. Zebra finches (*Taeniopygia guttata*) are highly gregarious, non-territorial, and socially monogamous, using their song solely for the purpose of mate attraction and pair maintenance. Only males sing, and preferentially use the song of their own father as a learning model. Zebra finches raised in isolation develop a song with abnormal properties, including unusual note structure and decreased stereotypy (Price, 1979; Williams, Kilander & Sotanski, 1993). Isolated males often fail to develop a canonical motif, and will only rarely repeat a given sequence of notes. Untutored songs also often include repeated notes, resembling the structure of the trills of canaries (Williams, 2004). While these abnormalities may arise due to the absence of a song model normally provided by a tutor, some features of untutored song appear to arise due to the absence of behavioral feedback from conspecifics.

The zebra finch sensory period lasts from approximately 20-65 days of age, while the sensorimotor period lasts from days 35-90 (Brainard & Doupe, 2000),

though young finches deprived of social interaction during the sensitive period will continue to be able to learn for at least several weeks beyond the normal close of the sensitive period (Clayton, 1987; Eales, 1985). Zebra finches require minimal exposure to the tutor song, and can learn to sing well with less than a minute of interactive tutoring per day (Tchernichovski et al., 1999). Sensory responses to songs are traditionally thought to be fixed and immutable, but are increasingly understood to be modulated by prior experience (Gilbert et al., 2009; Thompson & Gentner, 2010). Neural responses to songs are strongly modulated by whether or not they are reinforced by food or social feedback, and differences in acquired salience predict learning rate (Bell et al., 2015). Throughout song development, zebra finches are naturally exposed to a highly social environment, which favors a function for listeners in song learning. In the gregarious brown-headed cowbird, female cowbirds selectively respond to immature male vocalizations with a non-vocal signal, in the form of a rapid lateral wing movement called a 'wing stroke'. Juvenile males attend to these cues, which are believed to be indicators of female arousal, and repeat elements which elicited a wing stroke, allowing female listeners to direct the course of song learning (West & King, 1988). Similar mechanisms may influence learning in zebra finches which, like cowbirds, are highly gregarious and experience a high degree of overlap in the sensory and sensorimotor phases of song learning (Roper & Zann, 2006; Slater et al., 1988), allowing the opportunity for social feedback to influence learning. As with buntings, canaries, and starlings, for zebra finches interaction with a live tutor leads to more effective song learning than passive exposure to a tape-recorded song (Chen et al., 2016; Deregnaucourt et al., 2013; Eales, 1989). The salience of adult

tutor song is based on physical proximity of the tutor (Mann & Slater, 1995), aggression directed towards the fledglings (Clayton, 1987; Jones & Slater, 1996), the tutor's mating status and partner quality (Eales, 1987; Mann & Slater, 1994), visual cues such as color morph (Mann, 1991; Mann & Slater, 1995), and auditory information such as song similarity between the father and subsequent song tutors (Clayton, 1987).

Juvenile males preferentially learn to sing from their fathers, even when other potential tutors are available, although they will learn from alternative tutors depending on the level of parental care they receive (Williams, 1990). Zebra finches cross-fostered under Bengalese finches (*Lonchura striata*) will produce a good copy of their foster-parent's song, even if a zebra finch model is available in a neighboring cage (Bohner, 1983; Immelmann, 1969). Price (1979) hand-reared zebra finches such that they imprinted on him, and then tutored them each time he fed them by playing an adult song from a tape-recorder hung around his neck. The finches learned only a few syllables from the recording. However, if a finch can control the delivery of a recorded song by pressing a key, causing presentation of the model to be contingent on their own actions, they can learn to produce a good imitation (Adret, 1993). Control over the stimulus, much like interaction with a live tutor, may increase the young bird's attention to the song, leading to better learning. Simply pairing a stimulus with the sound of the model might sufficiently enhance motivation or arousal to improve learning, as in the case of common nightingales (*Luscinia megarhynchos*) which will only learn a taped song when they can observe the researcher operating the loudspeaker (Todt et al., 1979). Furthermore, male siblings have an effect on song

learning, as multiple male zebra finches raised together by the same father will develop a highly variable song compared to that learned by a male without siblings (Tchernichovski & Nottebohm, 1998).

As in the brown-headed cowbird, non-singing female listeners are also known to affect song learning in the zebra finch (Jones & Slater, 1993). Males raised with deaf adult females sing more frequently and develop more atypical songs than those raised with hearing females (Williams, 2004), and blindfolded males raised with a tutor develop more accurate song when also raised with a female sibling than without one (Adret, 2004). These cases of enhanced learning in the presence of conspecifics may be the result of heightened arousal or attention in social contexts (ten Cate, 1991), or the result of attendance to song-elicited conspecific behaviors (Vyas et al., 2009). A recent discovery shows that zebra finch females may guide juvenile male song learning in a manner very similar to that seen in cowbirds, by selectively responding to more mature, complex, or arousing elements with a wing stroke (Carouso-Peck, Menyhart, DeVoogd & Goldstein, submitted). These movements are extremely rapid, lasting less than 0.3 seconds and imperceptible to the human eye, only visible when video-recorded and then played back at 30% speed. This may explain the failure of earlier efforts to determine what cues may be responsible for differing trajectories of juvenile song learning in the presence of females; past studies observed live zebra finches at real speed, such that their rapid cues could not be detected (e.g., Houx & ten Cate, 1998). This bias towards using human perceptual capacities to observe avian interactions has led to many interesting behaviors being overlooked in the past. Among the manakins, a South American group of birds known for their spectacular

courtship displays, the black manakin (*Xenopipo atronitens*), was thought to have a simple and lackluster display, with a courtship routine consisting only of repetitive hopping (Kirwan & Green, 2012). However, when the display of the black manakin was captured on high-speed video and slowed down, it was discovered that every "hop" was a very rapid (360 ms) and technically complex backwards summersault (see Lindsay et al., 2015). But if these movements are too rapid for humans to perceive, might they also be too quick for birds to perceive, much less use as a social cue to alter their own behavior? The golden-collared manakin (*Manacus vitellinus*) also has a very fast courtship display, which consists of mechanical sounds and rapid lateral leaps between sapling trunks. High-speed video revealed that prior to each leap, the male quickly flares his neck feathers into a 'beard', an action that takes an average of 53 milliseconds (Fusani et al., 2007). The timing of this beard-up motion has the highest rate of inter-individual variability of any aspect of the complex display, and is also the primary basis upon which females decide whether or not to copulate with a given male (Lainy Day, personal communication). At least in some avian species, individuals are able to both perceive and make behavioral alterations based on extremely rapid movements of conspecifics, far too fast for a human researcher to perceive unaided, as human visual system critical flicker fusion rate is about half that of a small bird (Healy et al., 2013).

Song Control Circuitry in a Social Brain

Until recently, social behavior in the brain was thought to be divided into

distinct nodes, each of which was the center for a particular category of social behavior, such as parental care, territoriality, or pair-bonding. An alternative model proposed by Sarah Newman (1999) instead suggested a social system network, a tightly interconnected system of limbic areas across which social behavior and motivation are distributed. Social behaviors are not localized to a particular area, but rather neural activity distributed in a certain way across the network generates a given behavior. Exactly what stimulus is necessary to elicit a behavior and how it manifests in the brain varies by species, sex, age, and life history traits such as gregariousness and territoriality. This social circuit overlaps significantly with the circuitry governing motivation and reward, in particular the amygdala, which mediates motivational arousal. The connection between the amygdala and ventral tegmental area (VTA) makes up much of the mesolimbic dopamine pathway modulating the behavioral response to rewarding or motivating stimuli (Syal & Finlay, 2011).

Before delving into the neurobiology underlying song learning and production, it is helpful to conceptualize the tasks the brain must accomplish in order to drive vocal learning. First, it must generate motor commands to the vocal organ (the syrinx). It must also modify these commands in response to auditory feedback (i.e. the bird detecting that its own song is not a match to its memorized model) or social feedback (i.e. behavior from a conspecific updating the bird's mental model of ideal song). This requires the brain to use feedback to evaluate song performance, then alter motor output to minimize the difference between the song and the ideal model (Mooney, 2009). Finally, the brain must motivate the bird both to sing and to adjust its song based on feedback, requiring some form of reward resulting from singing behavior and

accurate matching responses to auditory and social feedback. How the brain accomplishes the comparison between song output and the mental model of ideal song is still being investigated, but the neural mechanisms for song production and variability are better understood. Exploration of the neural circuitry underlying song behavior, plasticity, and variability may shed light on how this machinery incorporates social feedback into song learning.

Song behavior and learning is regulated by an interconnected network of discrete brain nuclei referred to as the song system, which distinguishes the songbird brain from that of birds which do not learn to vocalize (Kroodsma & Konishi, 1991; Wild, 2004). During song learning, these nuclei undergo anatomical and neurochemical changes (Alvarez-Buylla & Kirn, 1997). This network is composed of two pathways: the song motor pathway (SMP) and the anterior forebrain pathway (AFP), which together affect vocalizations through the muscles of the respiratory system and the syrinx (Figure 2.2). The SMP is a posterior motor pathway connecting nucleus RA (robust nucleus of the arcopallium), HVC (proper name, not an acronym; previously ‘high vocal center’), and nXIIts (tracheosyringeal portion of the 12th cranial nerve). Each of the precise individual functions of these regions is a matter of some debate, as discussed below, but together these connected regions control song production and some aspects of song learning. Lesions in the SMP will disrupt or entirely abolish singing (Simpson & Vicario, 1990). In contrast, the AFP is involved in evaluation of the bird’s song via auditory feedback and adaptive modification of the song, and is essential to both song learning and recognition (Brainard & Doupe, 2000). Lesions to this pathway will not immediately degrade crystallized song, but will

prevent accurate vocal learning by reducing song variability and plasticity (Bottjer et al, 1984; Olveczky et al., 2005). The AFP is an anterior cortical-basal ganglia-thalamic loop originating in HVC, which then projects to Area X of the paraolfactory lobe and LMAN (lateral magnocellular nucleus of the anterior neostriatum), ultimately connecting back to the motor pathway at RA (Doupe et al., 2005). Nuclei in the AFP, as well as its connections to the SMP, regress substantially by the time the sensitive period closes (Hermann & Arnold, 1991; Iyengar et al., 1999). The linkage between these two pathways, as well as the fact that both contain neurons which respond both to song production (Leonardo & Fee, 2005; McCasland 1987) and auditory or social stimulation (Margoliash, 1983; Vicario & Yohay, 1993; Yanagihara & Hessler, 2006), suggests a mechanism by which social feedback in response to a juvenile's song may influence vocal output.

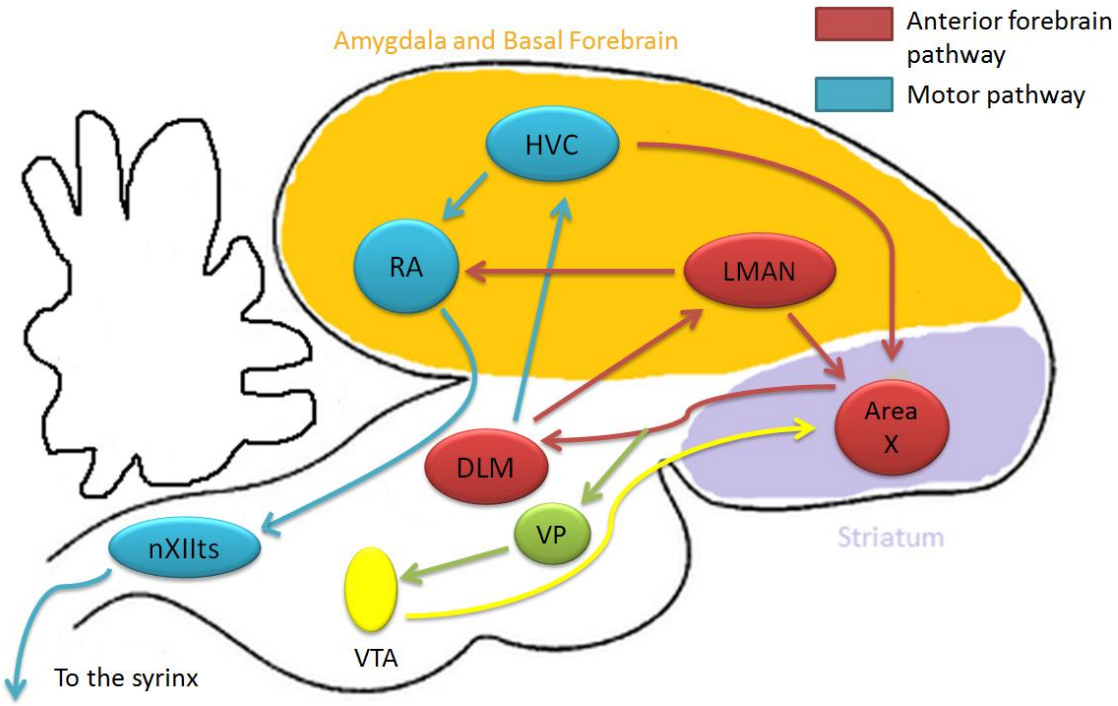


Figure 2.2. New thinking on the neural basis for birdsong. The song production pathway (motor pathway) consists of projections from DLM (dorso-lateral division of the medial thalamus) -> HVC -> RA -> nXIIts (indicated with blue arrows). The song learning pathway (anterior forebrain pathway) consists of connections between HVC, LMAN, Area X, DLM and RA (indicated with red arrows). Area X receives dopaminergic projections from VTA (yellow arrow). Area X indirectly projects song-related information back to VTA via the ventral pallidum (VP, green arrows). Based on Syal and Finlay's (2011) concept that brain areas generally believed to be homologous to mammalian pallium (neocortex) more closely resemble amygdala and basal forebrain (in orange) and areas considered homologous to basal ganglia more closely resemble striatum (in purple). This is based on the observation that bird vocal nuclei are located in tissue derived from lateral and ventral pallida, which gives rise to motivational/social circuitry in mammals. HVC; RA, robust nucleus of the arcopallium; LMAN, lateral magnocellular nucleus of the anterior nidopallium; DLM, medial nucleus of the dorsolateral thalamus; VTA, ventral tegmental area; nXIIts, tracheosyringeal portion of the nucleus hypoglossus.

The Song Motor Pathway: The Vocal Generator

A shared characteristic of human speech and birdsong, but not the majority of other animal vocalizations, is that they are controlled by the telencephalon. In birds, the anatomical basis of this control is the SMP. The nucleus HVC is a target for auditory and motor pathways, and conspicuously a shared component of the SMP and AFP. The size of HVC is also altered by social factors, as birds placed in a complex social environment develop a larger HVC than those housed with a single conspecific (Lipkind et al., 2002). This differential growth occurs despite the fact that birds in the simple social context sing far more than those in the complex context, indicating that it is caused not by vocal output levels but instead by the task of processing a rich auditory environment (Adar et al., 2008). HVC's position as a nexus connecting various circuits in the sensorimotor system makes it a good place to begin investigating song circuitry in social context.

HVC seems to function as a neural clock, firing in time with the elements of the song and generating its tempo. Singing-related activity in the SMP propagates through the system, arising in HVC prior to RA (McCasland, 1987). HVC firing activity is time-locked to individual syllables, but given that stimulation of HVC disrupts song (Ashmore et al., 2005) and that HVC activity is present even in deaf birds (McCasland & Konishi, 1981), it seems to serve a strictly motor rather than auditory function. HVC neurons projecting to RA rarely fire an action potential unless the bird sings, and even then the firing is very brief (about a 10 millisecond burst at a single point during a 1 second motif) (Hahnloser et al., 2002). Ablation of HVC neurons projecting to RA, but not those projecting to Area X, will severely degrade

the structure of the song (Scharff et al., 2000). This indicates that motor commands from HVC proceed directly to RA without passing through the AFP. Different neurons fire at different time points in the motif, suggesting that these neurons function to specify the production timing of different song elements. Given that some of the neurons also fire during intervening gaps of silence, they may also specify the timing of inter-note temporal spacing. In line with the idea that HVC controls song tempo, when HVC is cooled down the tempo of all aspects of the song, from individual notes to the entire motif, slow down by about 3% per degree Celsius of cooling (Long & Fee, 2008). Surprisingly, cooling has little effect on any other aspects of the song, such as amplitude or pitch. Cooling RA has little discernable effect on any aspect of song. It is possible that RA simply serves to turn HVC's timing signal into a motor signal, specifying the acoustic features of the song (like the structure of syllables) which should be produced according to the timing HVC specifies.

The Anterior Forebrain Pathway: Learning and Variation

As previously mentioned, the effect on song of lesioning components of the AFP is dependent on the developmental time at which it occurs. After song has crystallized, AFP lesions seem to have little immediate effect on song in most contexts. Lesions during song learning, however, prevent normal adult song from being fully learned, instead resulting in song with abnormally high stereotypy which never progresses beyond that point, as if premature crystallization has occurred (Scharff & Nottebohm, 1991). Neural activity in the AFP during singing is strongly modulated by the presence of a conspecific listener. The magnitude and variability of

activity in LMAN and Area X are lower and more consistent during singing directed to a female than undirected singing produced when the male is not oriented toward another conspecific (Hessler & Doupe, 1999a). LMAN seems to be the song's 'jitter injector', inserting variability into song during sensorimotor learning, thereby ensuring that the juvenile bird explores its acoustic range (Kao & Brainard, 2006). Stimulation of LMAN during singing will cause perturbation of the song, while LMAN inactivation reduces the bout-to-bout variability of plastic song (Olveczky et al., 2005), resulting in a repetitive and stereotyped song. The firing rate of LMAN neurons changes over developmental time, with their highest rate occurring during sensorimotor learning, suggesting that developmental change in song variability is a direct result of changes in LMAN activity. Supporting this idea, stimulating LMAN alters song structure almost immediately (as early as 30 milliseconds after stimulation) (Kao et al., 2005). LMAN was once thought to mediate song plasticity based on auditory feedback of the bird's own song as it attempted to match the song 'template' – the mental representation of the precise form of the memorized song of the tutor – yet LMAN neurons are entirely unresponsive to manipulated auditory feedback, suggesting that in LMAN the bird's own song is not used for error detection (Leonardo, 2004). Much like RA, LMAN serves a motor function, as neural activity in LMAN increases during song production (Hessler & Doupe, 1999b) and persists in deafened birds. Localized cooling of LMAN, much like HVC, slows down the timescale of subsong (Aronov et al., 2011). The timing signal from HVC, coupled with the 'noise' added to the signal from LMAN, may work in concert to deliver a precise motor pattern to the vocal muscles via RA.

It remains unclear whether LMAN is simply acting permissively to allow vocal plasticity, or if it is truly providing an instructive signal by injecting noise. Despite the differing level and timing of activity in LMAN between directed and undirected singing, the average pattern of firing for an individual neuron is similar across these social contexts (Kao et al., 2008). Furthermore, stimulating a single locus of LMAN will consistently change a targeted syllable in the same way, for example always increasing its pitch, rather than inserting variability at random (Kao et al., 2005). Rather than simply driving variation, LMAN may be systematically biasing acoustic output, instructively driving vocalizations toward a particular goal. When a finch is negatively reinforced by a burst of white noise in response to a particular syllable exceeding a certain pitch threshold, the bird will shift the syllable's pitch downwards (Sober & Brainard, 2009; Tumer & Brainard, 2007). Inactivation of LMAN will cause the syllable to instantly revert to its original pitch (Andalman & Fee, 2009). LMAN thus appears to be actively biasing song away from vocal errors.

Although the influence of the AFP on song is more obvious during song learning, it continues to regulate song variability in adults. After song crystallization, AFP activity and acoustic variability are higher during undirected song than directed song (Jarvis et al., 1998; Sossinka & Bohner, 1980), with more variable spike timing during undirected song (Kao et al., 2008). Lesioning LMAN will abolish this social-context-dependent variability (Kao & Brainard, 2006), but does not prevent a male bird from performing other courtship-related behaviors normally produced only in the presence of a female, such as dancing and beak wiping. Because males seem to be able to interpret female social cues in the absence of LMAN, their capacity to detect and

respond to social context must lie elsewhere in the brain and selectively activate LMAN when a female is not present.

The role of Area X in song learning remains as mysterious as its cryptic name implies, with conflicting findings thus far. Neurons in Area X exhibit highly variable patterns of firing during singing, leading some investigators to suggest that they may drive variability downstream in LMAN (Goldberg et al., 2010). Conversely, and in contrast to lesions of LMAN, juveniles with Area X lesioned exhibit normal vocal variability (Goldberg & Fee, 2011; Sohrabji et al., 1990). However, eliminating Area X leads to protracted variability in adult song, with abnormal acoustic structure and little resemblance to the song of the tutor (Scharff & Nottebohm, 1991). It has also been proposed that Area X is the site where the song template is stored and compared to the bird's own song output. This 'AFP comparison hypothesis' posits that auditory information about the bird's own song is transmitted to Area X, where it is evaluated against the template (Mooney, 2004; Sakata & Brainard, 2008). If this is the case, Area X neurons should respond to vocal errors while birds are singing, but distorted auditory feedback does not elicit such responses (Kozhevnikov & Fee, 2007; Leonardo, 2004). Furthermore, singing-related activity in Area X is not altered by deafening the bird (Hessler & Doupe, 1999a), contrary to what one would expect if the region was sensitive to perceived auditory error. The AFP comparison hypothesis is motivated largely by observations of AFP activation in response to auditory stimuli in birds while not singing, anesthetized, or asleep (Dave & Margoliash, 2000; Doupe, 1997; Prather et al., 2008). However, response to auditory input is ubiquitous throughout both the AFP and SMP in non-singing birds, even in syringeal motor

neurons, and is not a special property of Area X (Fee & Goldberg, 2011; Williams & Nottebohm, 1985). These observations led Fee and Goldberg (2011) to hypothesize that Area X does not store the song template, evaluate match to tutor, process auditory feedback, or receive an error evaluation signal from elsewhere in the AFP. Rather, Area X may receive an evaluation signal conveying the quality of song as it is produced via neuromodulatory inputs. Particularly well suited to carry such a global, rapid (<100 milliseconds), and time-dependent signal indicating good or bad vocal performance is the dopaminergic system, as discussed below.

Reward Value of Song: Plugging in to Social Circuitry

A great deal of effort has been made to map out which neural circuits are involved in various social behaviors such as sexual behavior, aggression, and parental behavior. Studies of these regions have often led to the unexpected conclusion that there is considerable overlap in the circuitry required for these behaviors, leading to exploration of the possibility that they form an integrated social behavior network, much like the song learning network. Newman (1999) proposed a system in mammals consisting of six limbic areas, each identified as regulating multiple social behaviors, and each reciprocally connected to each of the others (Figure 2.3). Rather than a single region regulating a single social behavior, each region responds to a number of stimuli. Social context leads to a distinct pattern of activation across regions, and this determines behavioral response. Evidence increasingly suggests that this network exists in all vertebrates, and some of the most relevant findings come from birds (see

Goodson, 2005), with network responses to social stimuli differently patterned in species of songbird with different levels of sociality (Goodson et al., 2005). The social behavior network is also reciprocally connected to the mesolimbic reward system, enabling social decision-making, which requires evaluation of the salience of a given stimulus before a behavioral response is executed (O'Connell & Hofmann, 2011). In order to determine the neural mechanisms by which social feedback may be affecting the trajectory of song learning, we must establish a) that singing is rewarding, activating the mesolimbic reward system, b) that social context modulates this reward value, and c) that the social-motivation system is connected to the song system and modulates its activity.

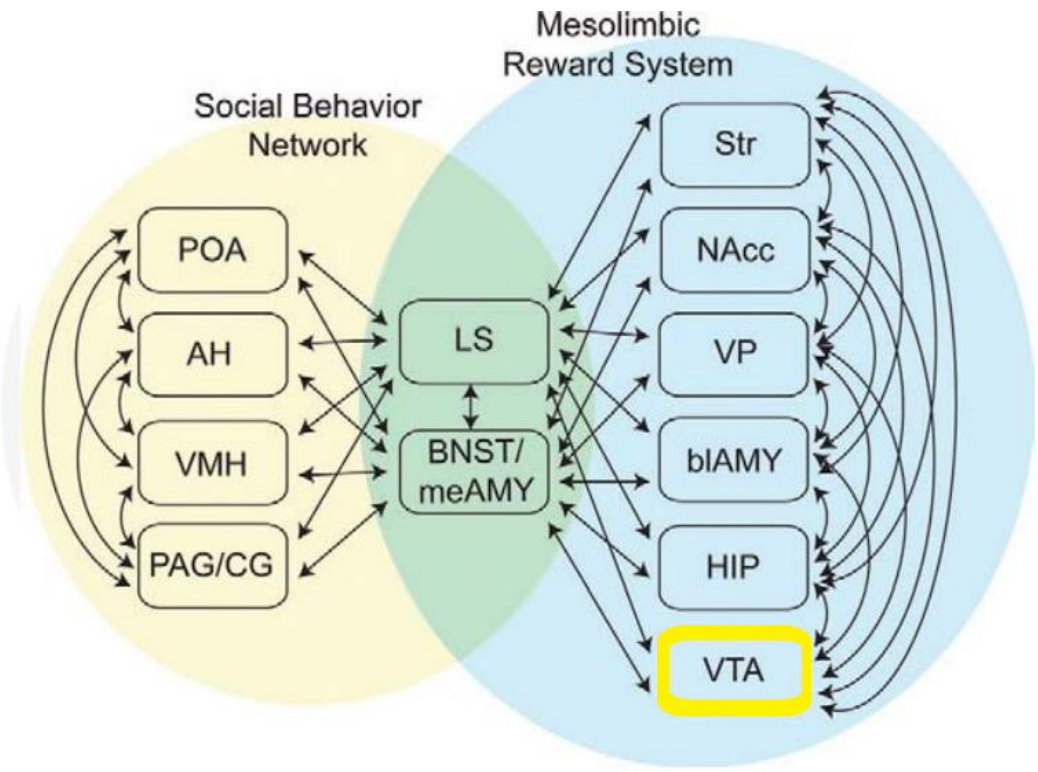


Figure 2.3. Interactive nodes of the networks regulating social decision making. By permission from O'Connell and Hoffmann (2011). Brain regions in the social behavior network (left) and mesolimbic reward center (right) as well as those involved in both systems (center) are shown. VTA has been highlighted yellow to indicate the region by which social/motivational centers project to the song learning system (as seen in Figure 2.2). Arrows indicate anatomical connections between systems in mammals. AH, anterior hypothalamus; blAMY, basolateral amygdala; BNST/meAMY, bed nucleus of the stria terminalis/medial amygdala; HIP, hippocampus; LS, lateral septum; NAcc, nucleus accumbens; PAG/CG, periaqueductal gray/central gray; POA, preoptic area; Str, striatum; VMH; ventromedial hypothalamus; VP, ventral pallidum; VTA, ventral tegmental area.

We know that song learning and singing behavior are controlled by the neural song system, and that both are affected by social factors. However, we know little about how social reward and song circuitry are linked. Reward associated with certain behaviors can act as a powerful incentive to perform those behaviors, and can influence food intake, copulation, and social interaction (Agmo & Berenfeld, 1990; Berridge & Kringelbach, 2008). For a socially gregarious species like the zebra finch,

motivation to seek social affiliation is important for survival, attention to social feedback during development is necessary for learning a reproductively successful song, and attention to social context in adult males is vital for attracting a mate. Given that songbirds are motivated to produce song at high rates in multiple social contexts, it is likely that singing is linked to reward. In humans, adults exhibit robust fMRI activation in the ventral striatum – a region involved in reward processing – when successfully learning new words, suggesting that language learning is intrinsically rewarding (Ripolles et al., 2014). The idea that vocalization is intrinsically rewarding has also been investigated in a non-oscine bird, the ring dove (*Streptopelia risoria*), in which male courtship involves cooing to a female. Estrogen then acts on the midbrain song nucleus (mICo) of the female, inducing her to coo in response (Cohen & Cheng, 1981). The female's coo, not the male's, causes an endocrine cascade in the female which results in egg-laying (Cheng, 2003). In order to investigate whether song is intrinsically rewarding in songbirds, Riters and Stevenson (2012) used a conditioned place preference paradigm to assess the reward value of singing directed (at a social partner) versus undirected song. When placed in an apparatus with two distinctive sides, male zebra finches preferred to spend time on the side where they had previously produced undirected song, suggesting that singing is coupled with reward state. They displayed no preference for the side of the apparatus in which they had previously sung directed song. This indicates that the role of reward in song production differs depending on social context, with directed and undirected song relying on different mechanisms of reward. Directed song is likely externally reinforced by conspecifics, with the associated reward value resulting from successful

social feedback elicitation, mate attraction, or copulation. In line with this hypothesis, males that produced directed song but failed to attract a female developed an aversion to the side of the apparatus where they had sung. Riters and Stevenson suggest that production of undirected song, without immediate social reinforcement, may instead rely on an intrinsic reward system and the act of producing undirected song could activate neural reward systems. However, in light of work suggesting that undirected song also serves a communicative purpose for more distal recipients, this hypothesis may need to be revisited (Dunn & Zann, 1996). What mechanisms might underlie the reward value of song, and how is it modulated in different social contexts?

A leading candidate for the cause of context-dependent neuronal activity in the AFP is dopamine, a catecholamine neurotransmitter and an important contributor to the neural mechanisms allowing animals to pursue reward (Koob, 1996). Goal-directed, socially motivated vocal behaviors, such as ultrasonic vocalizations in rats anticipating a social reward, can be stimulated by dopamine (Wintink & Brudzynski, 2001). In songbirds, dopamine plays a primary role in sexually motivated song directed towards females (Heimovics et al., 2009), and peripheral injections of dopamine agonists stimulate song produced in response to the introduction of a female, whereas antagonists inhibit song (Rauceo et al., 2007; Schroeder & Riters, 2006). Song produced in a social context appears to be highly rewarding, as elevated dopamine levels in the striatum of birds during directed singing resemble those after drug administration in mammals (Sasaki et al., 2006). The neural song system is strongly innervated by catecholaminergic neurons (Appeltants et al., 2001; Liao et al., 2013), which is not seen in comparable forebrain areas in bird species which do not

sing (Moons et al., 1994). Catecholaminergic innervation of the song system is also much stronger in male zebra finches than in non-singing females (Bottjer, 1992).

Dopamine also contributes to behavioral reinforcement that mediates appetitive learning (Panksepp & Moskal, 2008). Dopaminergic neurons in the VTA (a mesolimbic region) of monkeys trained on an operant task encode discrepancies between the expected reward normally delivered to them following a conditioned stimulus, and whether or not the reward is actually delivered (Schultz et al., 1993). Intense social interactions also result in increased glutamate activity in VTA (Huang & Hessler, 2008). In the zebra finch, EGR-1 expression in catecholaminergic neurons in VTA is significantly higher in birds which have been tutored socially than in untutored and passively tutored birds, suggesting that it is social interaction, not merely hearing song, that leads to activity in VTA (Chen et al., 2016). In songbirds, VTA is a primary source of dopaminergic input to both LMAN and Area X (Gale & Perkel, 2006; Lewis et al., 1981), where it also regulates synaptic plasticity (Ding & Perkel, 2004) and may encode prediction errors in song production. VTA neurons are known to exhibit singing-related activity, and projections from VTA to the song system modulate early gene activity related to social context (Hara et al., 2007). Dopamine levels in Area X are elevated more during directed song than undirected song (Sasaki et al., 2006), and infusion of dopamine antagonist near Area X (though possibly also affecting LMAN) increases variability during directed song (Leblois et al., 2010), hinting that dopamine may function as a regulator of AFP activity. Given that more than 95% of Area-X projecting VTA neurons are dopaminergic (Person et al., 2008), changes in VTA activity likely affect the release of dopamine in the AFP,

leading to changes in song output and variability. When perceived song quality is distorted with auditory feedback, VTA neuron activity is repressed, encoding this performance error (Gadagkar et al., 2016). Therefore, when a bird makes a vocal 'mistake' which does not match the memorized tutor song, or fails to elicit a wing stroke or other positive feedback from a conspecific, VTA neurons may detect this error and modulate song away from it. This idea is supported by the finding that lesions of dopaminergic inputs to Area X greatly impair vocal learning in the Bengalese finch, while having no detectable effect on vocal performance (Hoffmann et al., 2016).

Particularly among neuroscientists, song learning and the reward value driving it is considered strictly internally computed, the sole result of the young bird comparing its vocal output to its memorized template. However, just as monkeys can detect errors and learn to correct them for an external reward of juice, songbird vocal learning can be guided by external factors. A recent study found that spiking activity in Area X neurons was modulated by food rewards and reward signals in an operant task, however the authors concluded the role of Area X in general learning to be "limited and vestigial" (Seki et al., 2014). In contrast, we believe that the contribution of Area X to song learning is vital, and it may be the region that allows external social stimuli to affect song. Area X is highly sensitive to social context, and exhibits a marked, consistent, and rapid-onset response in electrophysiological activity when a female is introduced (Hessler & Doupe, 1999a). Several studies also suggest that Area X is primarily driving song learning rather than production, as the influence of the AFP on motor output is reduced in adults singing stable songs compared to juveniles

singing plastic songs (Bottjer et al., 1984; Scharff & Nottebohm, 1991; Sohrabji et al., 1990). Such differing level of activation in different social contexts may reflect a varying level of arousal, or could be specifically related to the communicative function of singing to another bird. In cowbirds, juveniles actively monitor conspecific listeners (West & King, 1988), and it seems probable that zebra finches are doing the same. Area X, via dopaminergic input from VTA neurons, may be responsible for altering the song in response to social feedback. It may also send song-related information back to VTA via the ventral pallidum, creating a two-way path between socially modulated song learning and reward value. Females have been shown to greatly prefer the song of their mate over the song of other conspecifics (Woolley & Doupe, 2008), suggesting that females are most aroused by song elements similar to those of their mate, resulting in maternal wing strokes that may influence song learning.

In order for rapid social signals to precisely affect the song learning trajectory by targeting specific syllables, Area X would need to receive information on both the precise time in the song at which feedback was received, and the current variability and structure of the song. Area X receives input from HVC in timed bursts which are brief and precisely locked to one time-point in the song with precision on the submillisecond scale (Kozhevnikov & Fee, 2007). This demonstrates that Area X receives a sparse and precise representation of the current time in the song (Fee & Goldberg, 2011), which could be used for Area X to generate a signal to drive variability in LMAN at a specific moment in the song sequence. LMAN also projects indirectly to Area X via axon collaterals in RA, which enables every neuron in LMAN driving vocal variability to be directly “observed” by Area X (Bottjer & Sengelaub,

1989; Vates et al., 1997). Together, this would allow Area X to receive a social reward signal via VTA neurons in response to external feedback, identify the precise time in the song at which the feedback was received, accordingly alter the level of song structure variability at that time-point, and then send this information back to social reward and motivation centers. This hypothesis has never been directly tested, as the role and form of social feedback in zebra finches is only just being discovered, and no mechanisms of socially guided vocal learning have been investigated at the neural level in this species.

Conclusions: Social-Motivational Learning in Context

Behavioral similarities between birdsong and human speech are matched by parallels in the neural system (Bolhuis et al., 2010; Doupe & Kuhl, 1999). Both share a neural dissociation between brain regions involved in the production and learning of vocalizations on the one hand, and in auditory memory and perception on the other (Bolhuis et al., 2012; Gobes & Bolhuis, 2007). Speech and language in humans involves Broca's area and associated regions in the frontal lobe, while perception and memory involve Wernicke's area and temporal lobe areas (Bolhuis et al., 2010). Human language is thought to be dependent on the cortex, however language often develops even in cases of severe cortical damage or complete loss of either the left or right cortical hemisphere (Bates et al., 2001). While catastrophic damage to cortical and sensory systems may leave language unscathed, any alteration to motivational systems proves extremely detrimental (Syal & Finlay, 2011). Until recently, the avian

song nuclei were thought to be homologous to mammalian cortical domains (Jarvis et al., 2005), however recent embryological evidence suggests avian vocal areas are limbic (Medina, 2007). As previously discussed, in mammals the limbic areas such as the amygdala and basal forebrain give rise to circuitry involved in social motivation. Placing song learning circuitry regions in areas associated with social reward (Figure 2.2) opens the possibility that vocal learning is directly coupled with social motivation, and that similar processes may underlie human language learning (Syal & Finlay, 2011).

Virtually all behavioral systems that incorporate learning of any sort are driven by a motivational context. The motivation and social circuits of the brain are inextricably connected, predisposing gregarious organisms to attach reward value to social partners. All that is required for socially guided vocal learning to occur is for evolution to lead to the connection of the social-motivation system to the vocal learning system. If song circuitry is indeed homologous to the basal forebrain and amygdala – regions intimately connected to social-motivational circuitry – rather than the neocortex as traditionally presumed, we must use this new perspective to seek homology to songbirds in other vocally learning organisms. Another commonly studied socially guided vocal learner, and potentially equally excellent a model organism for birds as birds are for them, is the human infant. Just as zebra finches can learn from a taped song only when played contingent on their own key pressing (Adret, 1993) and grey parrots fail to learn from non-interactive vocal models (Pepperberg, 1999), human infants are dependent on response contingency to develop mature vocalizations (Goldstein & Schwade, 2008). Infants are sensitive to social

contingencies from a young age, and demonstrate varying levels of sensitivity to contingency depending on the general responsiveness of their caregivers (Bigelow & Rochat, 2006).

In species that have evolved socially guided vocal learning, a unique link has been forged between social circuitry and vocal learning systems, such that learning is driven by social motivation. The 'social gating hypothesis' was first advanced in work on human infant language acquisition, proposing that language is gated by the motivating properties of social interaction such as attention and arousal (Kuhl, 2007). It has long been known that human parents alter their behavior when interacting with infants, most noticeably changing the prosody of their speech to generate *infant-directed speech*. Compared to adult-directed speech, infant-directed speech is higher in pitch and contains longer pauses, more repetition, and shorter utterances (Fernald et al., 1989), and more effectively attracts and sustains infant attention (Kuhl et al., 2005; Locke, 1993). It was recently found that adult zebra finches alter the structure of their vocalizations when interacting with juveniles in a manner strikingly similar to human infant-directed speech. When singing to a juvenile, adults lengthen the intervals between motifs, increase goodness of pitch, and repeat more introductory notes before song. Juveniles were also significantly more attentive to this 'pupil-directed' song than to undirected song, and those which received a greater proportion of pupil-directed song during development learned better matches to tutor song (Chen et al., 2016). This presents the intriguing possibility that adult finches could be actively teaching song to juveniles, and that, as in human parents and infants, shared attention between tutor and pupil drives vocal learning. Young zebra finches quickly shift the pitch of their song to

match that of a movie of an adult tutor facing towards them, but not one facing away from them (Ljubičić et al., 2016). As with zebra finches learning song, human infants learn from caregiver responses which are contingent on their own vocalizations, be they vocal (a tutor song or a spoken word) or non-vocal (a wing stroke or a smile) (Goldstein et al, 2003; Goldstein & Schwade, 2008). Infants also fail to learn the phonemic contrasts of a foreign language unless they are presented by a live, interactive tutor (Kuhl et al., 2003). Learning in both infants and songbirds may be gated by shared attention and social motivation, a process potentially enabled by similar neural circuitry linking vocal learning and social reward.

An ecologically valid and more complete understanding of vocal learning requires the incorporation of social factors. Social context and motivation affect the vocal learning system at virtually every level, both behaviorally and neurally. In humans and zebra finches, normal learning fails to occur without social exposure, and moment-to-moment social feedback to immature vocalizations shapes and guides vocal learning. In the songbird brain, social exposure during development leads to growth of HVC, while social context affects activity levels in Area X, which receives dopaminergic input from regions involved in social reward and motivation. Future research efforts should focus on the effects of manipulation of social-motivational circuitry on social behavior, including sensitivity to social cues, and resulting effects on song learning outcomes.

REFERENCES

Adar, E., Lotem, A., & Barnea, A. (2008). The effect of social environment on singing behavior in the zebra finch (Taeniopygia guttata) and its implication for neuronal recruitment. *Behavioural Brain Research*, *187*, 178–184

Adret, P. (1993). Operant conditioning, song learning and imprinting to taped song in the zebra finch. *Animal Behaviour*, *46*, 149-59.

Adret, P. (2004). Vocal imitation in blindfolded zebra finches (Taeniopygia guttata) is facilitated in the presence of a non-singing conspecific female. *Journal of Ethology, 22,* 29-35.

Adret-Hausberger, M., Guttinger, H.R., & Merkel, F.W. (1990). Individual life history and song repertoire changes in a colony of starlings (*Sturnus vulgaris*). *Ethology*, *84*, 265-80.

Agmo, A., & Berenfeld, R. (1990). Reinforcing properties of ejaculation in the male rat: role of opioids and dopamine. *Behavioral Neuroscience, 104*, 177-82.

Akçay, C., Campbell, S.E., Reed, V.A., & Beecher, M.D. (2014). Song sparrows do not learn more song from aggressive tutors. *Animal Behaviour*, *94*, 151-159.

Alvarez-Buylla, A., & Kirn, J. R. (1997). Birth, migration, incorporation, and death of vocal control neurons in adult songbirds. *Journal of Neurobiology*, *33*, 585-601.

Andalman, A.S., & Fee, M.S. (2009). A basal ganglia-forebrain circuit in the songbird biases motor output to avoid vocal errors. *PNAS*, *106*, 12518–12523.

Appeltants D., Ball G.F., & Balthazart J. (2001). The distribution of tyrosine hydroxylase in the canary brain: demonstration of a specific and sexually dimorphic catecholaminergic innervation of the telencephalic song control nulcei. *Cell and Tissue Research*, *304*, 237–59.

Aronov D., Veit L., Goldberg J.H., & Fee M.S. (2011). Two distinct modes of forebrain circuit dynamics underlie temporal patterning in the vocalizations of young songbirds. *Journal of Neuroscience*, *31*, 3009 –3011.

Ashmore, R.C., Wild, J.M., & Schmidt, M.F. (2005). Brainstem and forebrain contributions to the generation of learned motor behaviors for song. *Journal of*

Neuroscience, *25*, 8543-8554.

Baptista, L.F. (1975). Song dialects and demes in sedentary populations of the white-crowned sparrow (*Zonotrichia leucophrys nuttali*). *University of California Publications in Zoology*, *105*, 1-52.

Baptista, L.F., & Gaunt, S.L.L. (1997). Social interaction and vocal development in birds. In C.T Snowdon & M. Hausberger (Eds.) *Social interaction influences on vocal development* (pp. 23-42). Cambridge: Cambridge University Press.

Baptista, L.F., & Petrinovich, L. (1984). Social interaction, sensitive phases and the song template hypothesis in the white-crowned sparrow. *Animal Behaviour*, *32*, 172–181.

Baptista, L.F., & Petrinovich, L. (1986). Song development in the white-crowned sparrow: social factors and sex differences. *Animal Behaviour, 34*, 1359-1371.

Bates, E., Reilly, J., Wulfeck, B., Dronkers, N., Opie, M., Fenson, J., … Herbst, K. (2001). Differential effects of unilateral lesions on language production in children and adults. *Brain and Language*, *79*, 223–265.

Beecher, M.D. (2016). Birdsong learning as a social process. *Animal Behaviour*, http://dx.doi.org/10.1016/j.anbehav.2016.09.001.

Beecher, M.D., Burt, J.M., O'Loghlen, A.L., Templeton, C.N., & Campbell, S.E. (2007). Bird song learning in an eavesdropping context. *Animal Behaviour, 73*, 929-935.

Beecher, M.D., Campbell, S.F., & Stoddard, P.K. (1994). Correlation of song learning and territory establishment strategies in the song sparrow. *PNAS, 91*, 1450-4.

Bell, B.A., Phan, M.L., & Vicario, D.S. (2015). Neural responses in songbird forebrain reflect learning rates, acquired salience, and stimulus novelty after auditory discrimination training. *Journal of Neurophysiology, 113*, 1480-1492.

Berridge, K.C., & Kringelbach, M.L. (2008). Affective neuroscience of pleasure: reward in humans and animals. *Psychopharmacology (Berl), 199*, 457-80.

Bigelow, A.E., & Rochat, P. (2006). Two-month-old infants' sensitivity to social contingency in mother–infant and stranger–infant interaction. *Infancy, 9*, 313–325.

Bohner, J. (1983). Song learning in the zebra finch (*Taeniopygia guttata*): Selectivity in choice of a tutor and accuracy of song copies. *Animal Behaviour, 31*, 231-237.

Bolhuis J.J., Okanoya K., & Scharff C. (2010). Twitter evolution: Converging mechanisms in birdsong and human speech. *Nature Reviews Neuroscience, 11*, 747–759.

Bolhuis, J.J., Gobes, S.M.H., Terpsta, N.J., den Boer-Visser, A.M., & Zandberger, M.A. (2012). Learning-related neuronal activation in the zebra finch song system nucleus HVC in response to bird's own song. *PLoS One, 7,* e41556.

Bottjer, S.W. (1992). The distribution of tyrosine hydroxylase immunoreactivity in the brains of male and female zebra finches. *Journal of Neurobiology, 24*, 51–69.

Bottjer S.W., & Sengelaub D.R. (1989). Cell death during development of a forebrain nucleus involved with vocal learning in zebra finches. *Journal of Neurobiology*, 20, 609 – 618.

Bottjer, S.W., Miesner, E.A., & Arnold, A.P. (1984). Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science, 224*, 901-903.

Brainard, M.S., & Doupe, A.J. (2000). Auditory feedback in learning and maintenance of vocal behaviour. *Nature Reviews Neuroscience, 1*, 31–40.

Brenowitz, E.A., Margoliash, D., & Nordeen, K.W. (1997). An introduction to birdsong and the avian song system. *Journal of Neurobiology, 33*, 485-500.

Catchpole, C.K., & Slater, P.J.B. (1995). *Bird Song: Biological Themes and Variations*. Cambridge: Cambridge University Press.

Chaiken, M., Bohner, J., & Marler, P. (1993). Song acquisition in European starlings (*Sturnus vulgaris*): A comparison of the song of live-tutored, untutored, and wild caught males. *Animal Behaviour, 46*, 1079-1090.

Changeux, J.P., Heidman, T., & Patte, P. (1984). Learning by selection. In P. Marler & H.S. Terrace (Eds.), *The Biology of Learning* (pp. 115-133). Berlin: Springer-Verlag.

Chen, Y., Matheson, L.E., & Sakata, J.T. (2016). Mechanisms underlying the social enhancement of vocal learning in songbirds. *PNAS, 113*, 6641-6646.

Cheng, M.F. (2003). Vocal self-stimulation: from the ring dove story to emotion-based vocal communication. *Advances in the Study of Behavior, 33,* 309–353.

Clayton, N. S. (1987). Song tutor choice in Zebra finches. *Animal Behaviour, 35,* 714-721.

Cohen, J., & Cheng, M.F. (1981). The role of the midbrain in courtship behavior of the female ring dove (Streptopelia risoria): evidence from radiofrequency lesion and hormone implant studies. *Brain Research, 207,* 279–301.

Dave A.S., & Margoliash D. (2000). Song replay during sleep and computational rules for sensorimotor vocal learning. *Science, 290,* 812–816.

Deregnaucourt, S., Poirier, C., Kant, A. V., Linden, A. V., & Gahr, M. (2013). Comparisons of different methods to train a young zebra finch (Taeniopygia guttata) to learn a song. *Journal of Physiology - Paris, 107,* 210-218.

DeWolfe, B.B., Baptista, L.F., & Petrinovich, L. (1989). Song development and territory establishment in Nuttal's white-crowned sparrows. *Condor, 97,* 376-89.

Ding L., & Perkel D.J. (2004). Long-term potentiation in an avian basal ganglia nucleus essential for vocal learning. *Journal of Neuroscience, 24,* 488 –494.

Doupe A.J. (1997). Song- and order-selective neurons in the songbird anterior forebrain and their emergence during vocal development. *Journal of Neuroscience, 17,* 1147–1167.

Doupe A.J., & Kuhl P.K. (1999). Birdsong and human speech: common themes and mechanisms. *Annual Review of Neuroscience,* 22, *567*–631.

Doupe, A.J., Perkel, D.J., Reiner, A., & Stern, E.A. (2005). Birdbrains could teach basal ganglia research a new song. *Trends in Neuroscience, 28,* 353–363.

Dunn, A.M., & Zann, R.A. (1996). Undirected song in wild zebra finch flocks: contexts and effects of mate removal. *Ethology, 10,* 529–539.

Eales, L.A. (1985). Song learning in zebra finches: some effects of song model availability on what is learnt and when. *Animal Behaviour, 33,* 1293-1300.

Eales, L. A. (1987). Song learning in female-raised zebra finches: another look at the sensitive phase. *Animal Behaviour, 35,* 1356-1365.

Eales, L. A. (1989). The influences of visual and vocal interaction on song learning in zebra finches. *Animal Behaviour, 37*, 507-508.

Fee, M.S., & Goldberg, J.H. (2011). A hypothesis for basal ganglia-dependent reinforcement learning in the songbird. *Neuroscience, 198*, 152-170.

Fernald, A., Taeschner, T., Dun, J., Papousek, M., de Boysson Bardies, B., & Fukui, I. (1989). A cross-language study of prosodic modifications in mothers' and fathers' speech to preverbal infants. *Journal of Child Language*, *16*, 477–501.

Fromkin, V., Krashen, S., Curtis, S., Rigler, D., & Rigler, M. (1974). The development of language in Genie: a case of language acquisition beyond the 'critical period'. *Brain and Language*, *1*, 81–107.

Fusani, L., Giordano, M., Day, L. B., & Schlinger, B. A. (2007). High-speed video analysis reveals individual variability in the courtship displays of male golden-collared manakins. *Ethology, 113*, 964-972.

Gadagkar, V., Puzerey, P.A., Chen, R., Baird-Daniel, E., Farhand, A.R., & Goldberg, J.H. (2016). Dopamine neurons encode performance error in singing birds. *Science, 354*, 1278-1282.

Gale, S.D., & Perkel D.J. (2006). Physiological properties of zebra finch ventral tegmental area and substantia nigra pars compacta neurons. *Journal of Neurophysiology*, *96*, 2295–2306.

Gilbert, C.D., Li, W., & Piesch, V. (2009). Perceptual learning and adult cortical plasticity. *Journal of Physiology*, 587, 2743–2751.

Gobes, S.M.H., & Bolhuis, J.J. (2007). Bird song memory: A neural dissociation between song recognition and production. *Current Biology, 17*, 789–793.

Goldberg, J.H., & Fee, M.S. (2011). Vocal babbling in songbirds requires the basal ganglia-recipient motor thalamus but not the basal ganglia. *Journal of Neurophysiology, 105*, 2729 –2739.

Goldberg, J.H., Adler, A., Bergman, H., & Fee, M.S. (2010). Singing-related neural activity distinguishes two putative pallidal cell types in the songbird basal ganglia: comparison to the primate internal and external pallidal segments. *Journal of Neuroscience, 30*, 7088 –7098.

Goldstein, M.H., King, A.P., & West, M.J. (2003). Social interaction shapes babbling: testing parallels between birdsong and speech. *PNAS, 100*, 8030–8035.

Goldstein, M.H., & Schwade, J.A. (2008). Social feedback to infants' babbling facilitates rapid phonological learning. *Psychological Science, 19*, 515–523.

Goodson, J.L. (2005). The vertebrate social behavior network: evolutionary themes and variations. *Hormones and Behavior, 48*, 11-22.

Goodson, J.L., Evans, A.K., Lindberg, L., & Allen, C.D. (2005). Neuro-evolutionary patterning of sociality. *Proceedings of the Royal Society B, 272*, 227–235.

Hahnloser, R.H., Kozhevnikov, A.A., & Fee, M.S. (2002). An ultra-sparse code underlies the generation of neural sequences in a songbird. *Nature, 419*, 65–70.

Hara, E., Kubikova, L., Hessler, N.A., & Jarvis, E.D. (2007). Role of the midbrain dopaminergic system in modulation of vocal brain activation by social context. *European Journal of Neuroscience, 25*, 3406-3416.

Harlow, H.F., & Harlow, M.K. (1962). Social deprivation in monkeys. *Scientific American, 207*, 136-146.

Healy, K., McNally, L., Ruxton, G.D., Cooper, N., & Jackson, A.L. (2013). Metabolic rate and body size are linked with perception of temporal information. *Animal Behaviour, 86*, 685-696.

Heimovics, S.A., Cornil, C.A., Ball, G.F., & Riters, L.V. (2009). D1-like dopamine receptor density in nuclei involved in social behavior correlates with song in a context-dependent fashion in male European starlings. *Neuroscience, 159*, 962–73.

Hessler, N.A., & Doupe, A.J. (1999a). Social context modulates singing-related neural activity in the songbird forebrain. *Nature Neuroscience, 2*, 209-211.

Hessler, N.A., & Doupe, A.J. (1999b). Singing-related neural activity in a dorsal forebrain-basal ganglia circuit of adult zebra finches. *Journal of Neuroscience, 19*, 10461–10481.

Hoffmann, L.A., Saravanan, V., Wood, A.N., He, L., & Sober, S.J. (2016). Dopaminergic contribution to vocal learning. *Journal of Neuroscience, 36*, 2176-2189.

Houx, B.B., & ten Cate, C. (1998). Do contingencies with tutor behavior influence song learning in zebra finches? *Behaviour, 5*, 599-614.

Huang, Y.C., & Hessler, N.A. (2008). Social modulation during songbird courtship potentiates midbrain dopaminergic neruosn. *PLoS One, 3*, e3281.

Immelmann, K. (1969). Song development in the zebra finch and other estrildid finches. In R.A. Hinde (Ed.), *Bird Vocalizations* (pp. 61-81). Cambridge: Cambridge University Press.

Iyengar, S., Viswanathan, S.S., & Bottjer, S.W. (1999). Development of topography within song control circuitry of zebra finches during the sensitive period for song learning. *Journal of Neuroscience, 19*, 6037–6057.

Jarvis, E.D., Scharff, C., Grossman, M.R., Ramos, J.A., & Nottebohm, F. (1998). For whom the bird sings: context-dependent gene expression. *Neuron, 21*, 775–788.

Jarvis, E.D., Gunturkun, O., Bruce, L., Csillag, A., Karten, H., Kuenzel, W.,... Butler, A. B. (2005). Avian brains and a new understanding of vertebrate brain evolution. *Nature Reviews Neuroscience, 6*, 151–159.

Jerne, N. (1967). Antibodies and learning: Selection versus instruction. In G. C. Quarton, T. Melnechuk & F.O. Schmitt (Eds), *The neurosciences, a study program* (pp. 200-205). New York: Rockefeller University Press.

Jones, A., & Slater, P. (1993). Do young male zebra finches prefer to learn songs that are familiar to females with which they are housed? *Animal Behaviour, 46*, 616-617.

Jones, A., & Slater, P. (1996). The role of aggression in song tutor choice in the zebra finch: Cause or effect? *Behaviour, 133*, 103-115.

Kao, M.H., & Brainard, M.S. (2006). Lesions of an avian basal ganglia circuit prevent context-dependent changes to song variability. *Journal of Neurophysiology, 96*, 1441–1455.

Kao, M.H., Doupe, A.J., & Brainard, M.S. (2005). Contributions of an avian basal ganglia-forebrain circuit to real-time modulation of song. *Nature, 433*, 638–643

Kao, M.H., Wright, B.D., & Doupe, A.J. (2008). Neurons in a forebrain nucleus

required for vocal plasticity rapidly switch between precise firing and variable bursting depending on social context. *Journal of Neuroscience*, *28*, 13232–13247.

Kirwan, G.M., & Green, G. (2012). *Cotingas and manakins*. Princeton, NJ : Princeton University Press.

Konishi, M. (1965). The role of auditory feedback in the control of vocalization in the white-crowned sparrow. *Zeitschrift fur Tierpsychologie*, *22*, 770-83.

Koob, G.F. (1996). Hedonic valence, dopamine and motivation. *Molecular Psychiatry*, *1*, 186–189.

Kozhevnikov, A.A., & Fee, M.S. (2007). Singing-related activity of identified HVC neurons in the zebra finch. *Journal of Neurophysiology*, *97*, 4271– 4283.

Kroodsma, D.E., & Miller, E.H. (1996). *Ecology and evolution of acoustic communication in birds.* Comstock, Ithaca, NY.

Kroodsma, D.E., & Verner, J. (1978). Complex singing behaviors among *Cistothorus* wrens. *Auk*, *95*, 703-716.

Kroodsma, D., & Konishi, M. (1991). A suboscine bird (eastern phoebe, Sayornis phoebe) develops normal song without auditory feedback. *Animal Behaviour*, *42*, 477–484.

Kuhl, P.K. (2007). Is speech learning 'gated' by the social brain? *Developmental Science*, *10*, 110–120.

Kuhl, P.K., Tsao, F.M., & Liu, H.M. (2003). Foreign-language experience in infancy: effects of short-term exposure and social interaction on phonetic learning. *PNAS*, *100*, 9096–9101.

Kuhl, P.K., Coffey-Corina, S., Padden, D., & Dawson, G. (2005). Links between social and linguistic processing of speech in preschool children with autism: behavioral and electrophysiological evidence. *Developmental Science*, *8*, F1–F12.

Lane, H.L. (1976). *The wild boy of Aveyron.* Cambridge, MA: Harvard University Press.

Lanyon, W. E. (1979). Development of song in the wood thrush (Hylocichla

mustelina) with notes on a technique for hand-rearing passerines from the egg. *American Museum Novitates, 2666*, 1–27.

Leblois, A., Wendel, B.J., & Perkel, D.J. (2010). Striatal dopamine modulates basal ganglia output and regulates social context-dependent behavioral variability through D1 receptors. *Journal of Neuroscience, 30*, 5730 –5743.

Lenneberg, E.H. (1967). *Biological foundations of language.* New York: Wiley.

Leonardo, A. (2004). Experimental test of the birdsong error-correction model. *PNAS, 101*, 16935–16940.

Leonardo, A., & Fee, M.S. (2005). Ensemble coding of vocal control in birdsong. *Journal of Neuroscience, 25*, 652–661.

Lewis, J.W., Ryan, S.M., Arnold, A.P., & Butcher, L.L. (1981). Evidence for a catecholaminergic projection to area X in the zebra finch. *Journal of Comparative Neurology, 196*, 347–354.

Liao, C., Wang, S., Pan, X., Hou, G., & Li, D. (2013). Dopamine modulates the excitability of projections neurons in the robust nucleus of the arcopallium in adult zebra finches. *PLoS One, 8*, e82497.

Lindsay, W.R., Houck, J.T., Giuliano, C.E., & Day, L.B. (2015). Acrobatic courtship display coevolves with brain size in manakins (Pipridae). *Brain, Behavior, and Evolution, 85*, 29-36.

Lipkind, D., Nottebohm, F., Rado, R., & Barnea, A. (2002). Social change affects the survival of new neurons in the forebrain of adult songbirds. *Behavioral Brain Research, 133*, 31–43.

Ljubičić, I., Bruno, J.H., & Tchernichovski, O. (2016). Social influences on song learning. *Current Opinion in Behavioral Sciences, 7*, 101-107.

Locke, J. (1993). *The path to spoken language.* Cambridge, MA: Harvard University Press.

Long, M.A., & Fee, M.S. (2008). Using temperature to analyze temporal dynamics in the songbird motor pathway. *Nature, 456*, 189–194.

Mann, N. I., & Slater, P. J. B. (1994). What causes young male zebra finches, *Taeniopygia guttata*, to choose their father as song tutor? *Animal Behaviour,*

47, 671-677.

Mann, N. I., & Slater, P. J. B. (1995). Song tutor choice by zebra finches in aviaries. *Animal Behaviour, 49*, 811-820.

Mann, N. I., Slater, P. J. B., Eales, L. A., & Richards, C. (1991). The influence of visual stimuli on song tutor choice in the Zebra finch, Taeniopygia guttata. *Animal Behaviour, 42*, 285-293.

Margoliash, D. (1983). Acoustic parameters underlying the responses of song-specific neurons in the white-crowned sparrow. *Journal of Neuroscience*, *3*, 1039–1057.

Marler, P. (1970). A comparative approach to vocal learning: Song development in white-crowned sparrows. *Journal of Comparative and Physiological Psychology*, *71*, 1-25.

Marler, P. (1991). Song-learning behavior: the interface with neuroethology. *Trends in Neuroscience, 14*, 199-206.

Marler, P., & Nelson, D.A. (1993). Action-based learning: A new form of developmental plasticity in bird song. *Netherlands Journal of Zoology*, *43*, 91-103.

Marler, P., & Peters, S. (1982). Developmental overproduction and selective attrition: new processes in the epigenesis of birdsong. *Developmental Psychobiology*, *15*, 369-78.

McCasland, J.S. (1987). Neuronal control of birdsong production. *Journal of Neuroscience, 7*, 23–39.

McCasland, J.S., & Konishi, M. (1981). Interaction between auditory and motor activities in an avian song control nucleus. *PNAS, 78*, 7815–7819.

Medina, L. (2007). Do birds and reptiles possess homologues of mammalian visual, somatosensory and motor cortices? In Jon H. Kaas (Ed.), *Evolution of nervous systems* (Vol. 2, pp. 163–194). New York: Academic Press.

Carouso-Peck, S. Menyhart, O., DeVoogd, T.J., & Goldstein, M.H. (submitted). Contingent parental responses guide zebra finch song learning in naturalistic social conditions.

Mooney, R. (2004). Synaptic mechanisms for auditory-vocal integration and the correction of vocal errors. *Annals of the New York Academy of Sciences, 1016*, 476 – 494

Mooney, R. (2009). Neural mechanisms for learned birdsong. *Learning and Memory, 16*, 655-669.

Moons, L., Van Gils, J., Ghijsels, E., & Vandesande, F. (1994). Immunocytochemical localization of L-DOPA and dopamine in the brain of the chicken (Gallus domesticus). *Journal of Comparative Neurology, 346*, 97–118.

Morris, D. (1954). The reproductive behaviour of the zebra finch (Poephila guttata), with special reference to pseudofemale behaviour and displacement activities. *Behaviour, 6*, 271–322.

Newman, S.W. (1999). The medial extended amygdala in male reproductive behavior: a node in the mammalian social behavior network. *Annals of the New York Academy of Sciences, 877*, 242–257.

Nice, M.M. (1943). Studies in the life history of the song sparrow II. The behavior of the song sparrow and other passerines. *Transactions of the Linnaean Society, 6*, 1-238.

Nottebohm, F. (1993). The search for neural mechanisms that define the sensistive period for song learning in birds. *Netherlands Journal of Zoology, 43***,** 193-234.

O'Connell, L.A., & Hofmann, H.A. (2011). The vertebrate mesollimbic reward system and social behavior network: a comparative synthesis. *Journal of Comparative Neurology, 519*, 3599-639.

Odom, K.J., Hall, M.L., Riebel, K., Omland, K.E., & Langmore, N.E. (2014). Female song is widespread and ancestral in songbirds. *Nature Communications, 5*, 3379.

Olveczky, B.P., Andalman, A.S., & Fee, M.S. (2005). Vocal experimentation in the juvenile songbird requires a basal ganglia circuit. *PLoS Biol*ogy, *3*, e153

Panksepp, J., & Moskal, J. (2008). Dopamine and seeking: Subcortical reward systems and appetitive urges. In A. J. Elliot (Ed.), *Handbook of approach and avoidance motivation* (pp. 67-88). New York: Taylor and Francis.

Payne, R. B., & Payne, L. L. (1997). Field observations, experimental design, and the time and place of learning bird song. In C. T. Snowdon & M. Hausberger (Eds.), *Social influences on vocal development* (pp. 57–84). Cambridge: Cambridge University Press.

Pepperberg, I.M. (1993). A review of the effects of social interaction on vocal learning in African grey parrots (Psittacus erithacus). *Netherlands Journal of Zoology, 43*, 104-24.

Pepperberg, I.M. (1999). *The Alex studies: Cognitive and communicative abilities of grey parrots.* Cambridge, MA: Harvard University Press.

Person, A.L., Gale, S.D., Farries, M.A., & Perkel, D.J. (2008). Organization of the songbird basal ganglia, including area X. *Journal of Comparative Neurology, 508*, 840-866.

Prather, J.F., Peters, S., Nowicki, S., & Mooney, R. (2008). Precise auditory-vocal mirroring in neurons for learned vocal communication. *Nature, 451*, 305–310.

Price, P. (1979). Developmental determinants of structure in zebra finch song. *Journal of Comparative and Physiological Psychology, 93*, 260−277.

Rauceo, S., Harding, C.F., Maldonado, A., Gaysinkaya, L., Tulloch, I., & Rodriguez, E. (2007). Dopaminergic modulation of reproductive behavior and activity in male zebra finches. *Behavioural Brain Research, 187*, 133-39.

Rice, J.O., & Thompson, W.L. (1968). Song development in the indigo bunting. *Animal Behaviour, 16*, 462-469.

Ripollés, P., Marco-Pallarés, J., Hielscher, U., Mestres-Missé, A., Tempelmann, C., Heinze, H., … Noesselt, T. (2014). The role of reward in word learning and its implications for language acquisition. *Current Biology, 24*, 1-6.

Riters, L.V., & Stevenson, S.A. (2012). Reward and vocal production: song-associated place preference in songbirds. *Physiology & Behavior, 106*, 87-94.

Roper, A., & Zann, R. (2006). The onset of song learning and song tutor selection in fledgling zebra finches. *Ethology, 112*, 458-470.

Sakata, J.T., & Brainard, M.S. (2008). Online contributions of auditory feedback to neural activity in avian song control circuitry. *Journal of Neuroscience, 28*, 11378-11390.

Sasaki, A., Sotnikova, T.D., Gainetdinov, R.R., & Jarvis, E.D. (2006). Social context-dependent singing-regulated dopamine. *Journal of Neuroscience, 26*, 9010–9014.

Scharff, C., & Nottebohm, F. (1991). A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. *Journal of Neuroscience, 11*, 2896-913.

Scharff, C., Kirn, J.R., Grossman, M., Macklis, J.D., & Nottebohm, F. (2000). Targeted neuronal death affects neuronal replacement and vocal behavior in adult songbirds. *Neuron, 25*, 481–492.

Schroeder, M.B., & Riters, L.V. (2006). Pharmacological manipulations of dopamine and opioids have differential effects on sexually motivated song in male European starlings. *Physiology & Behavior, 88*, 575–584.

Schultz, W., Apicella, P., & Ljungberg, T. (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *Journal of Neuroscience, 13*, 900–913.

Seki, Y., Hessler, N.A., Xie, K., & Okanoya, K. (2014). Food rewards modulate the activity of song neurons in Bengalese finches. *European Journal of Neuroscience, 39*, 975-983.

Seyfarth, R.M., & Cheney, D.L. (1986). Vocal development in vervet monkeys. *Animal Behaviour, 34*, 1640-1658.

Simpson, H.B., & Vicario, D.S. (1990). Brain pathways for learned and unlearned vocalizations differ in zebra finches. *Journal of Neuroscience, 10*, 1541–1556.

Slater, P. J. B., Eales, L. A., & Clayton, N. S. (1988). Song learning in zebra finches (Taeniopygia guttata): progress and prospects. *Advances in the Study of Behavior, 18*, 1-34.

Snowdon, C.T., & Hausberger, M. (1997). Introduction. In C. T. Snowdon & M. Hausberger (Eds.), *Social influences on vocal development* (pp. 1-6). Cambridge: Cambridge University Press.

Sober, S.J., & Brainard, M.S. (2009). Adult birdsong is actively maintained by error correction. *Nature Neuroscience, 12*, 927–931.

Sohrabji, F., Nordeen, E.J., & Nordeen, K.W. (1990). Selective impairment of song

learning following lesions of a forebrain nucleus in the juvenile zebra finch. *Behavioral and Neural Biology, 53*, 51-63.

Sossinka, R., & Bohner, J. (1980). Song types in the zebra finch Poephila guttata castanotis. *Zeitschrift für Tierpsychologie*, *53*, 123–132.

Syal, S., & Finlay, B.F. (2011). Thinking outside the cortex: social motivation in the evolution and development of language. *Developmental Science, 14*, 417-430.

Takahashi, D.Y., Fenley, A.R., & Ghazanfar, A.A. (2016). Early development of turn-taking with parents shapes vocal acoustics in infant marmoset monkeys. *Philosophical Transactions of the Royal Society B, 371*, 20150370.

Tchernichovski, O., & Nottebohm, F. (1998). Social inhibition of song imitation among sibling male zebra finches. *PNAS, 95*, 8951-8956.

Tchernichovski, O., Lints, T., Mitra, P.P., & Nottebohm, F. (1999). Vocal imitation in zebra finches is inversely related to model abundance. *PNAS, 96*, 12901–12904.

ten Cate, C. (1991). Behaviour-contingent exposure to taped song and zebra finch song learning. *Animal Behaviour, 42*, 857-859.

Thielcke, G. (1970). Learning of song as a possible pacemaker of evolution. *Zeitschrift fuer Zoologische Systematik und Evolutionsforschung, 8*, 309-320.

Thielcke, G. (1984). Gesangslernen beim Gartenbaumlaufer (*Certhia brachydactyla*). *Die Vogelwarte, 32*, 282-297.

Thompson, J.V., & Gentner, T.Q. (2010). Song recognition learning and stimulus-specific weakening of neural responses in the avian auditory forebrain. *Journal of Neurophysiology, 103*, 1785–1797.

Thorpe, W.H. (1958). The learning of song patterns by birds, with especial reference to the ong of the chaffinch *Fringilla coelebs. Ibis, 100*, 535-570.

Todt, D., & Geberzahn, N. (2003). Age dependent effects of song exposure: song crystalization sets a boundary between fast and delayed vocal imitation. *Animal Behaviour, 65*, 971-979.

Todt, D., Hultsch, H., & Heike, D. (1979). Conditons affecting song acquisition in nightingales (*Luscinia megarhynchos* L.). *Zeitschrift fur Tierpsychologie, 51*,

23-35.

Tumer, E.C., & Brainard, M.S. (2007). Performance variability enables adaptive plasticity of ‘‘crystallized’’ adult birdsong. *Nature*, 450, 1240–1244.

Van Hout, A.J.M., Pinxten, R., Darras, V.M., & Eens, M. (2012). Testosterone increases repertoire size in an open-ended learner. *Hormones and Behavior*, *62*, 563-568.

Vates, G.E., Vicario, D.S., & Nottebohm, F. (1997). Reafferent thalamo-“cortical” loops in the song system of oscine songbirds. *Journal of Comparative Neurology, 380*, 275–290.

Vicario, D.S., & Yohay, K.H. (1993). Song-selective auditory input to a forebrain vocal control nucleus in the zebra finch. *Journal of Neurobiology*, *24*, 488–505.

Vyas, A., Harding, C., Borg, L., & Bogdan, D. (2009). Acoustic characteristics, early experience, and endocrine status interact to modulate female zebra finches' behavioral responses to songs. *Hormones and Behavior*, *55*, 50-59.

Waser, M.S., & Marler, P. (1977). Song learning in canaries. *Journal of Comparative and Physiological Psychology, 91*, 1-7.

West, M.J., & King, A.P. (1988). Female visual displays affect the development of male song in the cowbird. *Nature, 334*, 244−246.

West, M.J., King, A.P., & Duff, M.A. (1990). Communicating about communicating: When innate is not enough. *Developmental Psychobiology, 23*, 585-598.

Wild, J.M. (2004). Functional neuroanatomy of the sensorimotor control of singing. *Annals of the New York Academy of Sciences, 1016*, 438–462.

Williams, H. (1990). Models for song learning in the zebra finch: fathers or others? *Animal Behaviour, 39*, 745-757.

Williams, H. (2004). Birdsong and singing behavior. *Annals of the New York Academy of Sciences, 1016*, 1-30.

Williams, H., Kilander, K., & Sotanski, M.L. (1993). Untutored song, reproductive success and song learning. *Animal Behaviour, 45*, 695−705.

Williams, H., & Nottebohm, F. (1985). Auditory responses in avian vocal motor

neurons: a motor theory for song perception in birds. *Science, 229*, 279-282.

Wintink, A.J., & Brudzynski, S.M. (2001). The related roles of dopamine and glutamate in the initiation of 50-kHz ultrasonic calls in adult rats. *Pharmacology, Biochemostry, and Behavior, 70*, 317–323.

Woolley, S.C., & Doupe, A.J. (2008) Social context-induced song variation affects female behavior and gene expression. *PLOS Biology, 6*, e62.

Yanagihara, S., & Hessler, N. A. (2006), Modulation of singing-related activity in the songbird ventral tegmental area by social context. *European Journal of Neuroscience*, *24*, 3619-3627.

Yasukawa, K., Blank, J.L., & Patterson, C.B. (1980). Song repertoires and sexual selection in the red-winged blackbird. *Behavioral Endocrinology and Sociobiology*, *7*, 233-238.

CHAPTER 3

FEMALE SOCIAL FEEDBACK REVEALS NON-IMITATIVE MECHANISMS OF VOCAL LEARNING IN ZEBRA FINCHES

Abstract

Learning of song in birds provides a powerful model for human speech development (Kuhl, 2003; Williams, 2004; Goldstein, King & West, 2003). However, the degree to which songbirds and humans share social mechanisms of vocal learning is unknown. Although it has been demonstrated as a vocal learning mechanism in human infants (Kuhl, 2006; Goldstein & Schwade, 2008; Albert, Schwade & Goldstein, 2017), learning via active social feedback is considered rare and atypical among non-human animals (West & King, 1988). We report here the first evidence that song learning in the zebra finch (*Taeniopygia guttata*), the most common model species of vocal learning and development, utilizes socially guided vocal learning. We demonstrate experimentally that the songs of juvenile zebra finches are guided towards mature vocal forms by real-time visual feedback from adult females that is contingent on their early, immature vocalizations. Using a video playback paradigm, we found that juvenile birds that received non-vocal female feedback contingently on their immature song learned significantly better and more accurate song than did yoked controls that received identical but non-contingent feedback. Both contingent and non-contingent groups sang at similar rates. Thus we have provided the first evidence suggesting that non-imitative social learning is a crucial, potentially widespread mechanism of vocal development, and have established a foundational

parallel between humans and our most ubiquitous animal model of vocal learning – the crucial role of social feedback to immature vocalizations in the development of communication.

Introduction

The role of social influences on vocal learning in nonhuman animals is poorly understood (Kuhl, 2003; Williams, 2004; Chen, Matheson & Sakata, 2016; Theofanopoulou, Boeckx & Jarvis, 2017), though social interactions are crucial for early speech learning in human infants (Kuhl, 2006; Goldstein & Schwade, 2008). Contingent parental responses to the immature vocalizing of prelinguistic infants facilitate the development of speech and the learning of phonological patterns (Goldsten & Schwade, 2008; Albert, Schwade & Goldstein, 2017). Infants who receive non-contingent feedback do not show vocal learning. In contrast to these findings in humans, the immature song of songbirds is generally considered a non-communicative epiphenomenon of motor practice (Marler, 1970; Petrinovich, 1972; Marler, 1997). Little attention has been paid to the social ecology of vocal learners, and few studies have addressed the potentially significant function of immature vocalizations in eliciting social feedback (West & King, 1988; Chen, Matheson & Sakata, 2016; Takahashi, Liao & Ghazanfar, 2017). Early vocal behavior may provide learning opportunities by exploiting information available in the immediate social environment. We investigated the ability of young songbirds to use social feedback to refine their vocal repertoires. Our approach extends traditional models that emphasize imitative learning via memorization and sensorimotor integration.

Traditional models of songbird vocal learning are based on classic work with sparrows: tutor vocalizations are first memorized, and later in development the learner attempts to match its vocalizations to the memorized template (Figure 3.1). Sparrows

show no evidence of using social feedback to construct their songs, though conspecific behavior plays a role in selecting among previously-learned songs (Marler & Nelson, 1992). In contrast, vocal learning in a phylogenetically distant songbird, the brown-headed cowbird (*Molothrus ater*) results from social feedback to immature vocalizations (West & King, 1988). Cowbirds are raised without access to adult song models, due to their reproductive strategy as brood parasites, depositing eggs into nests of other species. Juvenile male cowbirds develop more reproductively potent songs when exposed to non-singing female cowbirds. Females respond selectively to more mature male song elements produced, using a visual cue of a rapid lateral wing movement (a 'wing-stroke') (West & King, 1988). Juvenile males preferentially incorporate song elements which received wing-strokes into their final songs, resulting in songs more preferred by females. Although cowbirds are the only songbird species in which social cues have been experimentally demonstrated to guide vocal learning, they have not been used as a direct model of human speech acquisition, perhaps because as brood parasites they do not fit the traditional model of learning (Figure 3.1). Using social feedback to immature vocalizations to guide vocal development – known as *socially guided vocal learning* - is therefore currently considered a rare, atypical learning strategy among songbirds.

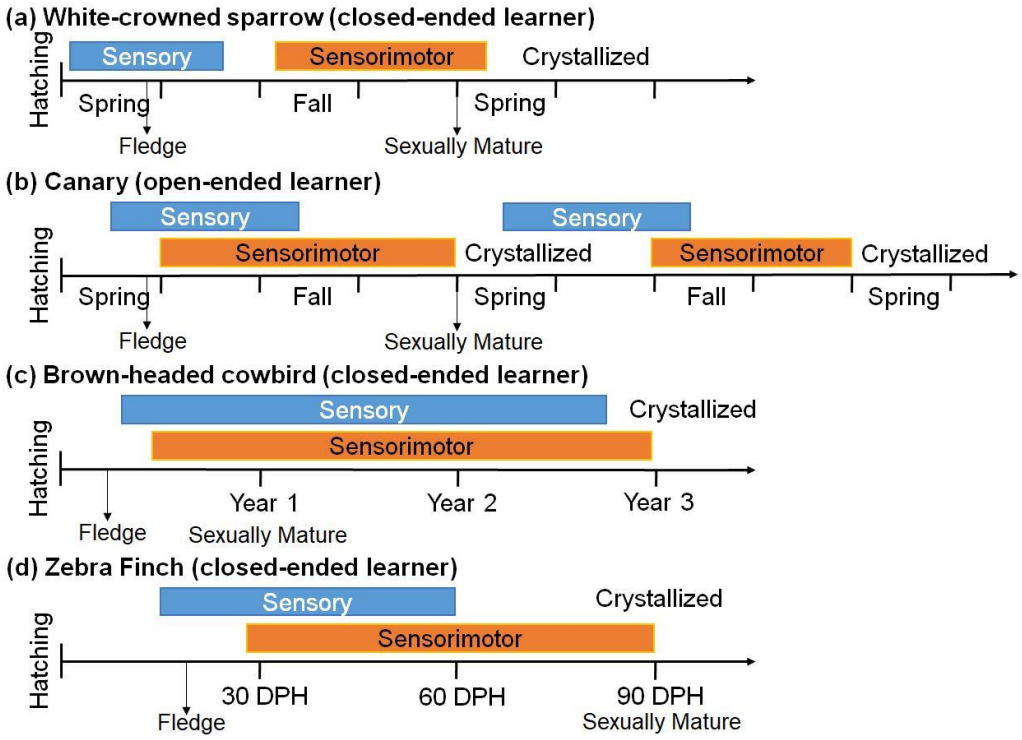


Figure 3.1. Four types of vocal learning trajectories in songbirds.
In many seasonally-breeding species (e.g. white-crowned sparrows), the sensory memorization phase and sensorimotor practice phase are temporally distinct. During learning, song is variable ('plastic'), but for closed-ended learners song ultimately 'crystallizes' into a stable form following sexual maturity. Open-ended learners (e.g. canaries) re-open their sensitive learning phase as adults, with sensory/sensorimotor overlap during each learning recapitulation. Although brown-headed cowbirds are closed-ended learners, song crystallization takes multiple years. The sensory and sensorimotor phases overlap, permitting social feedback to immature vocalizations to influence song production. Zebra finches show rapid development and sensory/sensorimotor phase overlap.

Zebra finches are the most common model species for human vocal learning, but unlike humans, are thought to acquire their learned vocalizations solely via imitation of a tutor. However, there is mounting evidence that social influences are crucial to zebra finch vocal development. Zebra finches require interaction with a live tutor to develop species-typical song (Williams, 2004; Eales, 1989; Derégnaucourt et al., 2013). They attend and learn better when song is presented by an interactive tutor,

even a heterospecific (Immelmann, 1969), or contingent on their own pressing of a key (Tchernichovski et al., 1999). Furthermore, visual cues are essential for normal vocal interaction (Perez et al., 2005) and song learning, as juveniles kept in visual isolation from conspecifics do not develop normal song even with acoustic interaction (Morrison & Nottebohm, 1992). The proximal mechanism driving improved song learning in the presence of a live tutor is unknown. Non-singing female listeners also affect song development, as males raised with deaf females sing more frequently and develop more atypical songs than those raised with hearing females (Williams, 2004), and blindfolded males develop more accurate song when raised with a female sibling (Adret, 2003). Despite such evidence of social influences on vocal development, socially guided vocal learning has never been experimentally demonstrated in zebra finches, or any non-parasitic songbird.

To determine whether zebra finches use socially guided vocal learning, and to discover what cues non-singing females may provide to males as they develop their song, we manipulated the timing of female responses to juvenile songs. We used video playback of a non-vocal female arousal behavior, presented contingently on juvenile zebra finch song production across vocal development, to influence song learning outcomes.

Results

Using video playbacks of female finches as stimuli enabled us to achieve precise experimental control over displays that could serve as social reinforcement. Zebra finches are known to learn from and sing to videos of conspecifics (Ikebuchi and Okanoya, 1999; Guillette & Healy, 2017). Subjects consisted of 9 pairs of

juvenile zebra finch genetic brothers raised by their respective parents until 35 days post-hatch (dph), the beginning of the sensorimotor song practice phase (Figure 3.2A), when each brother was placed in a sound attenuated chamber equipped with a video monitor and camera (Figure 3.2B), and randomly assigned to an experimental Contingent condition (CC) or Yoked control (YC) condition. For one hour daily for 25 days (Figure 3.2A), CC birds were video- and audio-monitored by an experimenter. Each time the bird sang, the experimenter triggered playback on the monitor of an adult female appearing and performing a 'fluff-up', consisting of erecting her feathers followed by high frequency side-to-side movements of the upper body (Figure 3.2C). Like the wing-strokes of cowbirds, fluff-ups are most commonly exhibited in response to complex, attractive song (Vyas et al., 2009). The video stimulus was shown to the CC subject immediately contingent on their own song production whenever an immature song was produced during the experimental period. The video stimulus appeared simultaneously on the monitors of the CC and YC male siblings, such that playback occurred contingently on the song production of CC birds but unrelated to YC birds' behavior. Thus CC and YC subjects received identical and simultaneous amounts of video stimulus presentation, but videos were not contingent on YC subjects' own song production.

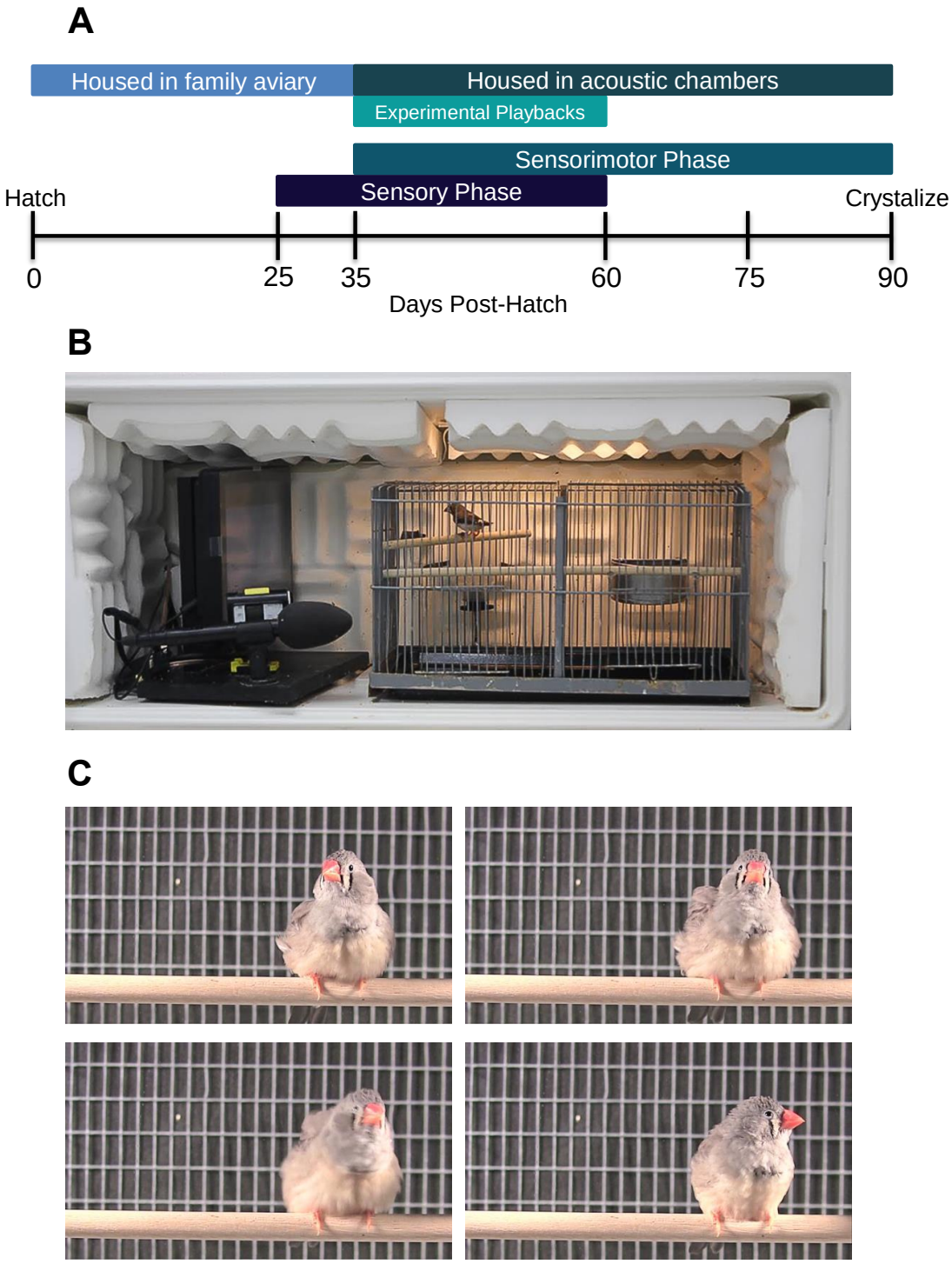


Figure 3.2. Experimental apparatus and stimulus for video playback study.
(A) Experimental timeline for all subjects, housed in family aviaries until relocation to acoustic chambers at 35 dph, the start of the sensorimotor period (Figure 3.1). Playbacks

occurred daily until the end of the sensory/sensorimotor overlap phase at 60 dph. Birds were kept in chambers until song crystallization and recording at 90 dph. (B) Sound attenuation chambers used for playbacks and recording (see *Methods*). (C) Stills from the fluff-up video stimulus shown to subjects, contingent on CC male song, sampled every 0.5 seconds. The video stimulus (3s total duration) viewed by subjects consisted of a life-sized adult female zebra finch fading into view on a perch over 0.5 seconds (top left), erecting body feathers over 0.6 seconds (top right), performing a 0.2 second fluff-up 'shake' (bottom right), then returning to a neutral position (bottom right) and fading from view.

Adult songs of all subjects were recorded after sexual maturity at 90 ± 1 dph (Figure 3.3A). To assess learning accuracy, songs of each sibling pair were acoustically compared to those of their shared genetic and social father using Sound Analysis Pro 2.0 (Tchernichovski et al., 2000). CC birds incorporated more of their father's song material into their motifs than YC birds, as indicated by significantly higher acoustic percent similarity to tutor song ($t(8) = 4.418$, $p = 0.002$) (Figure 3.3B). CC bird similarity outcomes ($M = 65.266$, $SD = 5.348$) were comparable to those of zebra finches raised with optimal levels of exposure to tutor song (Tchernichovski et al., 1999). Eight of the nine CC subjects outperformed their YC brothers in song similarity scores (Wilcoxon Signed Ranks $z = -2.547$, $p = 0.011$) (Figure 3.3C).

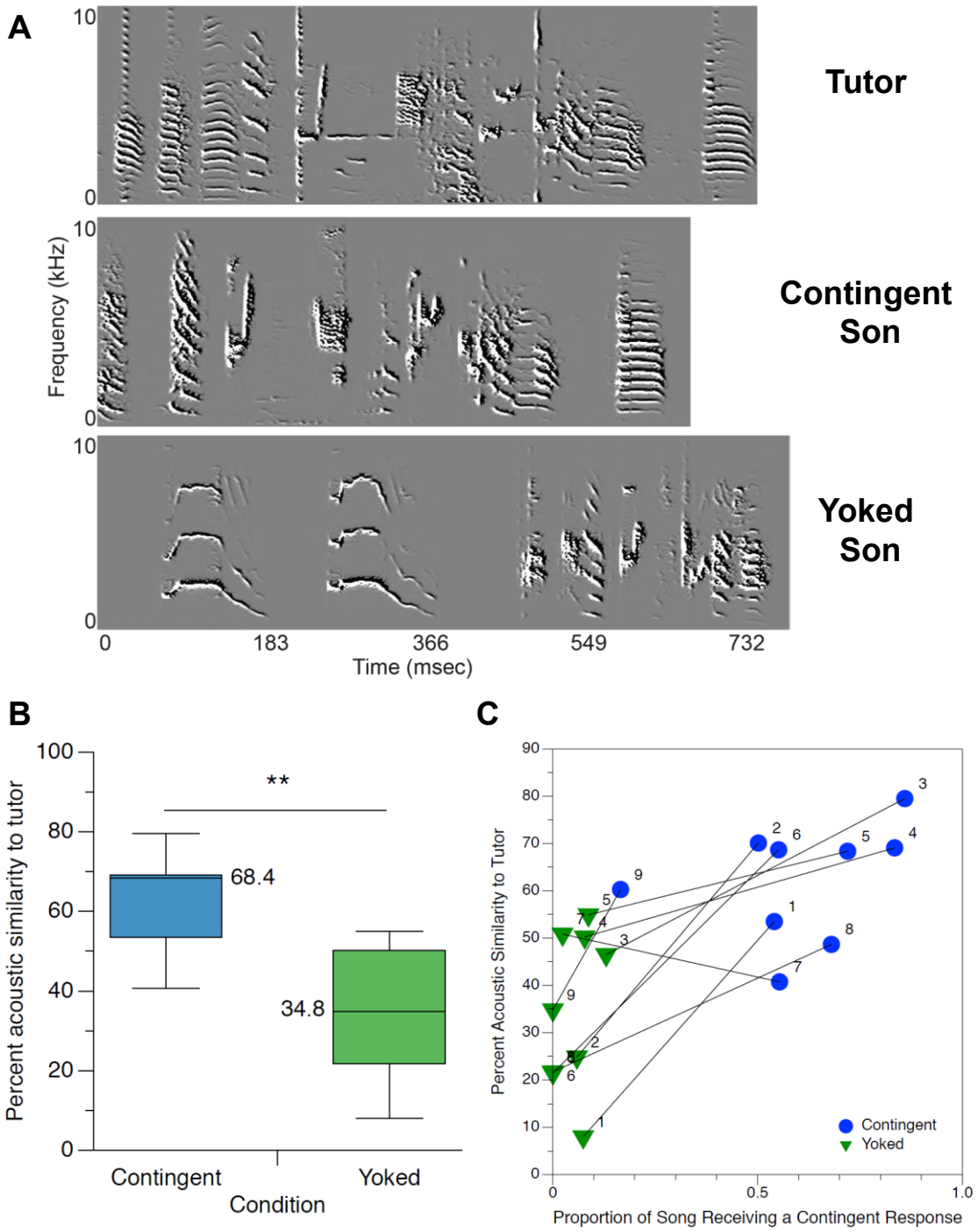
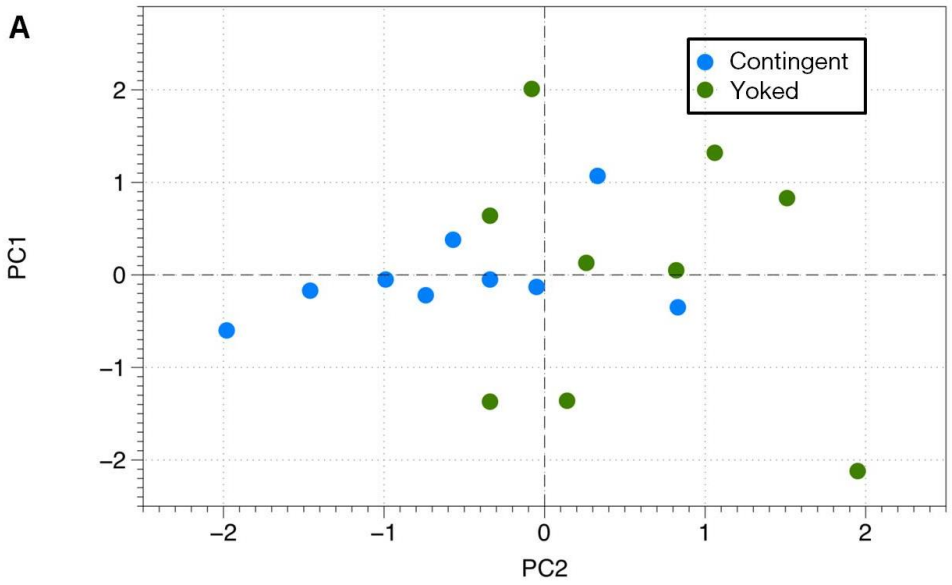


Figure 3.3. More accurate learning of tutor's crystalized song in subjects exposed to Contingent playback condition.
(A) Sample spectrograms of crystallized directed song of an adult tutor (top) and its two male offspring, recorded at 90dph. Subjects were raised with the tutor until 35dph and then exposed to the video playback procedure from 35 – 60dph. As seen in this Yoked spectrogram (bottom), YC birds produced atypical songs. Examples of spectrograms from all subjects are

shown in Figure 3.7B. (B) Percent acoustic similarity to tutor of crystallized song by group, Contingent (n = 9) and Yoked (n = 9) ($t(8) = 4.418$, $p = 0.002$, two-tailed paired t-test). Boxes indicate interquartile range. (C) Relation between final song percent similarity to tutor and proportion of subject-produced songs receiving a video playback within 1 second, Wilcoxon Signed Ranks $z = -2.547$, $p = 0.011$ ($n = 9$). Lines connect sibling pairs of CC and YC subjects. Sibling pairs are indicated by pair number.

To analyze spectral features of songs, we performed a principle components analysis constructed from average whole-song pitch, frequency modulation, entropy, goodness of pitch, and amplitude modulation (see *Methods*). The first two components of the PCA had respective Eigenvalues of 1.826 and 1.586, and accounted for 36.533% and 31.72% (68.235% cumulative) of the total variance in song. We found significant differences in spectral structure of songs between CC and YC males using PC2 ($t(16) = 2.77$, $p = 0.014$) (Figures 3.4A and 3.4B). A Kolmogorov-Smirnov test revealed that YC subjects have a significantly broader distribution of entropy than CC subjects ($z = 1.414$, $p = .037$) (Table 3.1).

A



B

Spectral Feature	PC1	PC2
Goodness	.822	-.387
Frequency Modulation	.621	.220
Pitch	.606	.593
Entropy	.288	.741
Amplitude Modulation	-.561	.698

Figure 3.4. Differences in spectral acoustic features of crystalized song between Contingent and Yoked condition subjects.
(A) Principle component analysis calculated from spectral features of crystalized songs. Contingent and Yoked bird values on PC2, which accounted for 31.72% of variance, were significantly different ($t(16) = 2.77$, $p = 0.014$) (see *Methods*). (B) Weighting of spectral features included in each principle component (for values of each acoustic parameter, see Table 3.1).

Table 3.1. Summary of mean song acoustic parameter values in Contingent Condition and Yoked Control subjects.
Standard deviations are shown in parentheses.

Acoustic Parameter	CC Mean	YC Mean
Pitch	2.077 (.421)	2.309 (.348)
Frequency Modulation	1.883 (.160)	2.029 (.118)
Entropy	2.275 (.351)	3.162 (1.131)
Goodness	3.268 (.426)	3.426 (.531)
Amplitude Modulation	2.317 (.152)	2.398 (.422)

Several behavioral results also suggested differences in learning outcomes may have been caused by differing individual levels of interest in the video across development (Figure 3.5), indicating that contingent responses increased social feedback salience. Within the CC group, we found a trending positive correlation between average number of arousal behaviors per playback session and final overall similarity to tutor song ($r(8) = 0.660$, $p = 0.053$) (Figure 3.6A). Overall similarity was significantly higher for CC than YC subjects ($t(8) = 3.074$, $p = 0.015$) (Figure 3.6B).

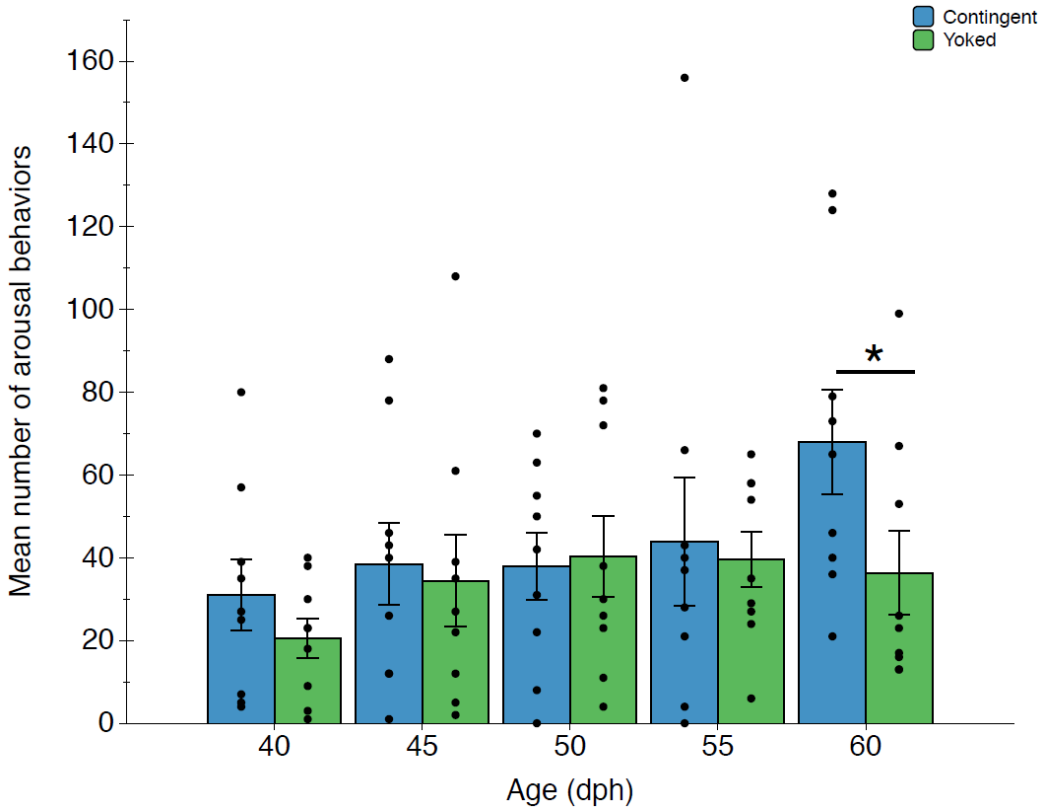


Figure 3.5. Differences in numbers of arousal behaviors during playback sessions between Contingent and Yoked birds emerged by 60 dph.
We conducted a 2 (Condition: CC, YC) x 5 (Age: 40, 45, 50, 55, 60dph) repeated measures ANOVA on mean number of arousal behaviors (fluff-ups and beak wipes). Neither main effect was significant. We found a significant Condition by Age interaction: $F(4, 32) = 4.68$, $p = 0.004$. Tests of simple main effects at each age revealed a significant difference between conditions only at 60dph ($F(1. 8) = 5.60$, $p = 0.045$). Error bars ± 1 S.E.

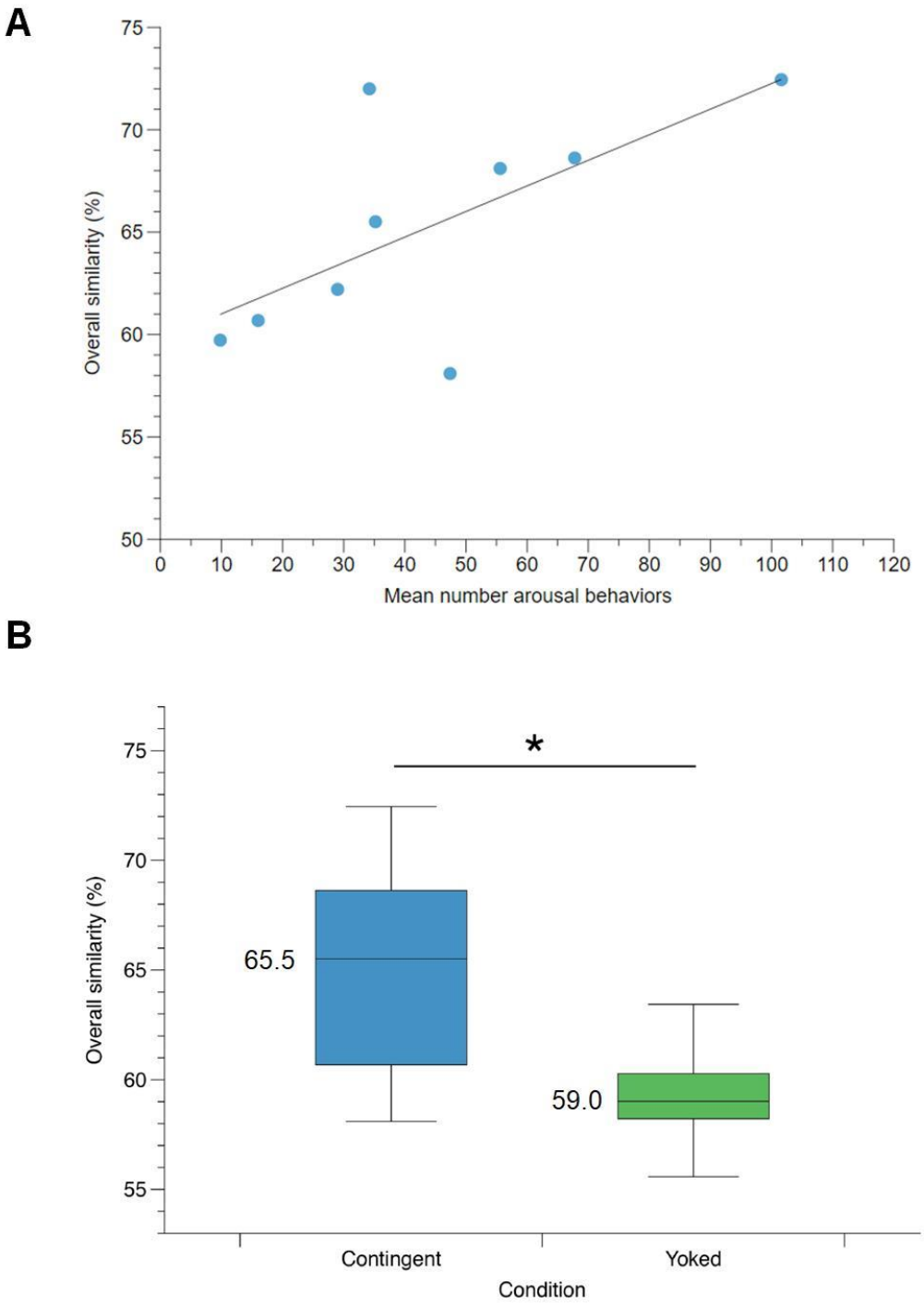


Figure 3.6. Correlation between arousal behaviors during playback sessions and final overall song similarity to tutor for Contingent Condition birds.
Overall similarity is a composite measure of percent similarity, accuracy, and sequential match to tutor song. (A) There was a positive trend in correlation ($r(8) = 0.660$, $p = 0.053$) for CC subjects between average number of arousal behaviors (fluff-ups and beak wipes) they exhibited across developmental time (35-60 dph) during experimental video playback periods,

and final overall similarity of adult crystalized song to tutor song at 90dph. (B) Significant difference in final overall acoustic song similarity to tutor between Contingent and Yoked subjects ($t(8) = 3.074$, $p = 0.015$). Similarity was measured at 90 dph.

Discussion

Our findings are the first demonstration of a proximal mechanism by which females affect male song development and facilitate socially guided vocal learning in the zebra finch. Juvenile finches which received contingent, non-vocal social feedback from a video of a female performing a fluff-up behavior learned their tutor's song significantly more accurately than a sibling which saw identical videos on a yoked schedule. Several previous studies have found that the presence of non-singing female zebra finches improves song learning in juvenile males (Williams, 2004; Adret, 2003), the mechanisms of which have never been explained. We hypothesize that contingent social feedback serves to reinforce the memorized representation of the song, indicating to the young learner the social potency of his attempts to reproduce the song. By receiving generalized positive feedback over the developmental period of song learning, the learner is likely motivated to continue his attempts at producing his memorized song. Such social motivation is known to facilitate song development (Theofanopoulou, Boeckx & Jarvis, 2017; Baran & Peck et al., 2017).

These results are also the first experimental demonstration that the song ontogeny of young male zebra finches can be significantly influenced by contingent visual displays from non-singing conspecifics. As human infants also use contingent social feedback to guide vocal development (Goldstein & Schwade, 2008; Albert, Schwade & Goldstein, 2017), our results provide evidence for a previously unknown

parallel between zebra finch song acquisition and human speech development. The presence of socially guided vocal learning in zebra finches suggests that this active form of learning, which most closely resembles human learning of speech, may be more common than previously thought. This study is a first step in elucidating socially guided vocal learning in the zebra finch, and reveals new avenues of research to elucidate proximal mechanisms of this learning strategy. To determine the importance of the form of the feedback, we are currently extending these findings using videos of female finches exhibiting wing-strokes, as well as non-biological stimuli.

In contrast to songbirds, the presence of socially guided vocal learning is better characterized in infant marmoset monkeys and humans, which both have the capacity for socially guided vocal learning. Young marmosets which receive more vocal feedback from parents contingent on their immature calls develop mature calls more quickly (Takahashi, Liao & Ghazanfar, 2017). Lack of parental interaction during development results in long-term disruptions to the acoustic structure of marmoset vocalizations, suggesting that parental feedback is necessary for proper vocal learning (Gultekin & Hage, 2018). Similarly, human infants rapidly learn to produce new phonological patterns in response to contingent reactions of caregivers, and their ability to learn phonological rules is not based on imitation (Goldstein & Schwade, 2008). Despite their phylogenetic distance, humans, marmosets, zebra finches, and cowbirds share life history traits that may have given rise to socially guided vocal learning as a solution to the problem of developing communicative competence. First, all three species are socially gregarious, ensuring developmental access to social feedback. Second, they use their learned vocalizations to facilitate and maintain social

bonds. Third, they can learn new vocal forms at the same time they are producing immature vocalizations (Figure 3.1), which may facilitate a role for social feedback in response to immature vocalizations as a means of influencing vocal learning (Zann, 1996). Furthermore, zebra finches and cowbirds are both non-territorial, and use song for attracting mates, meaning they benefit from attending to the song preferences of the opposite sex while developing a vocal repertoire.

Our finding of a novel mechanism that shapes vocal learning in the zebra finch offers a new approach to studying comparative vocal development, as it demonstrates that this ubiquitous model species learns from social contingencies as humans do, rather than solely from imitating previous auditory exposure. Why does social feedback have such a robust effect? The motivation and social circuits of the brain are inextricably connected, predisposing gregarious organisms to attach reward value to social partners (O'Connell & Hofmann, 2011). Socially guided vocal learning requires additional connections between the social-motivation system and the vocal learning system (Syal & Finlay, 2011). By placing the zebra finch, a species often studied in isolation, into a larger social context, we have uncovered new processes of non-vocal feedback that require investigation at neural and neuroendocrine levels of organization. Song learning is clearly affected by social factors, but how song system and social reward circuitry are linked is largely unknown. An emerging body of evidence indicates a strong role of nonapeptides such as arginine vasotocin on social motivation and song learning in zebra finches (Theofanopoulou, Boeckx & Jarvis, 2017; Baran & Peck et al., 2017). Learners perform real-time error correction in response to auditory feedback via dopaminergic connections between ventral

tegmental area (VTA) and Area X of the song system (Gadagkar et al., 2016). Producing song in a social context appears to be highly rewarding, as it elevates dopamine in the striatum of songbirds (Sasaki et al., 2006). Dopamine administration stimulates socially motivated vocalizations in songbirds (Heimovics et al., 2009), while dopamine antagonists inhibit vocal behavior (Rauceo et al., 2007). In the zebra finch, EGR-1 expression in catecholaminergic neurons in VTA is significantly higher in birds which have been tutored socially than in passively tutored birds, suggesting that social interaction, not merely hearing song, leads to activity in VTA (Chen, Matheson & Sakata, 2016). These seemingly disparate neuroendocrine findings can be integrated by our behavioral findings as part of a social feedback system that guides learning.

One reason for the lack of attention to social feedback as a driving force of song learning is that zebra finches can learn from taped song when it is triggered by their own key-pressing, though not if played to them passively (e.g. Tchernichovski et al., 1999). Key-pressing has long been exploited for vocal learning studies, but its efficacy has never been adequately explained. Our findings suggest that the contingency of social information (song) on the actions of the subject (key-pressing) in traditional paradigms may serve as an artificial proxy for social feedback. Studies in rodents often use key-pressing paradigms to measure social reward value or motivation, by having subjects press keys for access to conspecifics (e.g. Martin & Iceberg, 2015). Rather than allowing mice to passively experience a social stimulus, such paradigms require effort from subjects to obtain a social reward, potentially rendering it more salient. Similar studies in hamsters have found that subjects work

equally hard for social rewards as food rewards (Borland et al., 2017). Social feedback appears to be intrinsically rewarding across social species, causing increases in dopamine which mediate appetitive learning via behavioral reinforcement (Panksepp & Moskal, 2008). Social reinforcement only leads to learning when the reward is delivered contingently on the subject's own actions, as is the case for song stimuli in avian key-pressing paradigms.

In conclusion, we found that zebra finches, previously thought to learn only via imitation of an acoustic tutor, utilize the human-like learning strategy of socially guided vocal learning. Juveniles exposed to video playbacks of females performing a 'fluff-up' arousal behavior contingent on their own immature song production developed significantly more accurate songs than their genetic brothers exposed to identical, non-contingent feedback on a yoked schedule. Zebra finches, like cowbirds, are non-territorial and use their song solely for attracting mates, making integration of the preferences of the opposite sex into song a highly adaptive strategy for future reproductive success. Our finding of socially guided vocal learning in the zebra finch suggests the possibility that this learning strategy is more ubiquitous than previously imagined. Given the impact of social influences on zebra finch vocal development and its contrast with the classic model system of sparrows, we emphasize that there is no universal vocal learning strategy across songbird species (Brenowitz & Beecher, 2005), and the presence of socially guided vocal learning should be investigated across oscines. Incorporating social factors into studies of zebra finch learning will strengthen the species as a model system, as it will uncover new possibilities for drawing parallels with human speech acquisition. Because humans learn to speak via

selective social feedback to more advanced vocal forms, the discovery of a similar mechanism in zebra finches presents a new avenue of investigation for more accurately characterizing social mechanisms of human vocal development.

Methods

Subject Rearing and Housing

Subjects were eighteen male zebra finches hatched and raised in our laboratory's colony (n = 9 pairs). Parents were eight male and eight female unpaired adult zebra finches purchased from Magnolia Bird Farm in Riverside, California, placed in a large flight aviary (1.2 x 0.9 x 0.6 m) and allowed to pair and breed. All birds in the colony are marked with individually identifying colored leg bands. Birds were provided water, cuttle bone, and Kaytee Forti-Diet Finch Food ad libitum, with supplemental fresh spinach, carrots, peas, and hard-boiled egg every other day. Aviaries were equipped with plastic nest boxes lined with coconut fiber and additional loose fiber for nest-building material. Zebra finches are a monogamous, non-territorial, and highly gregarious estrildid finch species native to arid central Australia (Zann, 1996). They are commonly used in laboratory studies of vocal learning due to their simple song structures, rapid development, and ease of breeding in captivity.

Offspring were sexed visually at 30 days post-hatch (dph), when males first begin to develop orange cheeks and black-striped chests, which are absent in females. Provided a clutch contained at least two males, the eldest two males became experimental subjects. Offspring were raised in the aviary until 35 ± 1 dph, near the

beginning of the sensorimotor learning period when juvenile male zebra finches begin to produce practice song (Figure 3.1, Figure 3.2A), and coinciding with independence from parental feeding. At this time, brother pairs were removed from the communal aviary and placed in individual sound attenuated chambers. Because zebra finch chicks usually hatch on sequential days, brothers typically differed in age by one day. We randomized whether the older chick was placed in the Contingent (CC) or Yoked control (YC) condition. Sibling pairs raised simultaneously by the same parents were used to control for potential differences in parental behaviors, difficulty of and exposure to tutor song, and genetic effects. Subjects were housed in acoustic chambers until 90 dph (Figure 3.2A).

Ethical Note

All protocols were approved by and animal subject treatment in this study complied with the standards defined by the Cornell Institutional Animal Care and Use Committee. The study was designed to minimize number of birds used, disturbance, stress, and social isolation. No birds were sacrificed for this study, and following final song recordings all subjects were re-homed in other zebra finch labs.

Video Playback Apparatus

Sound attenuated chambers (104 x 48 x 43 cm) were internally lined with ‘Soundfoam M’ attenuating foam from Soundcoat Co Inc. Chambers. Each was equipped with two IKEA Dioder LED lights, on an automatic 12/12 light/dark

schedule. A LPH120 linear piston central air pump provided low airflow to all chambers. Cages within the chambers (46 x 44 x 36 cm) had cuttle bone, water, and seed provided *ad libitum*. At one end of each chamber was a wooden stand mounted with a Sony Actioncam HDR-AS15 digital HD video camera recording audio via a Sennheiser ME62/K6 omni-directional condenser microphone, a Morel MDT 29 speaker, and a 10.4" HP L6010 LED monitor (60 Hz refresh rate, 57% color gamut, 4:3 aspect ratio, 1024 x 768 resolution) overlaid with a 0.04 mm thick UV filter sheet allowing less than 10% transmission below 390 nanometers. This filter served to prevent excessive UV light produced by the monitors, detectable by the bird visual system but not by humans, from washing out the images on the monitor. The monitor was controlled by an iMac 21.5" 2.7GHz Quad-core Intel Core i5 desktop computer running Microsoft Powerpoint 2010. All audio recordings were run through a high-pass filter at 1100 Hz to exclude the low-frequency noise of the animal facility's air circulation system.

Experimental Schedule and Recording

Experimentation and recording occurred for one hour each day, beginning 30 minutes after artificial sunrise, from 36 ± 1 dph to 61 ± 1 dph, for 25 total days of recording per subject. During this time, an experimenter monitored the live video and audio from the chamber of the CC bird using an ActionCam wrist monitor and headphones connected to the microphone via an Alesis RA150 Stereo Power Amplifier. The experimenter triggered the video stimulus as soon as a CC bird transitioned from introductory notes into a core motif, which ensured playbacks only

occurred when song was produced. The video stimulus was 3 seconds long, showing a female zebra finch performing a fluff-up. New video playbacks were not triggered if the CC subject began singing a new song bout while the video was already playing. This resulted in an average of 60.1% of songs produced by CC birds receiving contingent feedback, while an average of 5% of songs produced by YC subjects received a chance contingent playback. For CC subjects, 81.3% of all contingent feedback videos overlapped with song production. In comparison, 36.5% of the videos that played contingently by chance for YC subjects overlapped with song.

Between sessions, when recordings and playbacks were not occurring, each chamber also housed a female canary as a social partner. Canaries share similar behaviors, diets, and size with zebra finches, but female canaries do not sing and are not attracted to zebra finch song, making them ideal as social partners which would not provide subjects with auditory or visual feedback on their song. To investigate whether final song outcomes were differentially influenced across groups by canary calls or social feedback, we performed an acoustic similarity analysis between the calls of our female canaries and the syllables of the crystalized songs of our subjects. We first recorded twenty canary calls from birds used in the study, and then used Sound Analysis Pro 2011 to perform similarity measures between the calls. We found that all canary calls were highly similar (>80%) to each other. We chose three calls with the greatest acoustic difference from each other to act as exemplars in further analyses (Figure 3.7A).

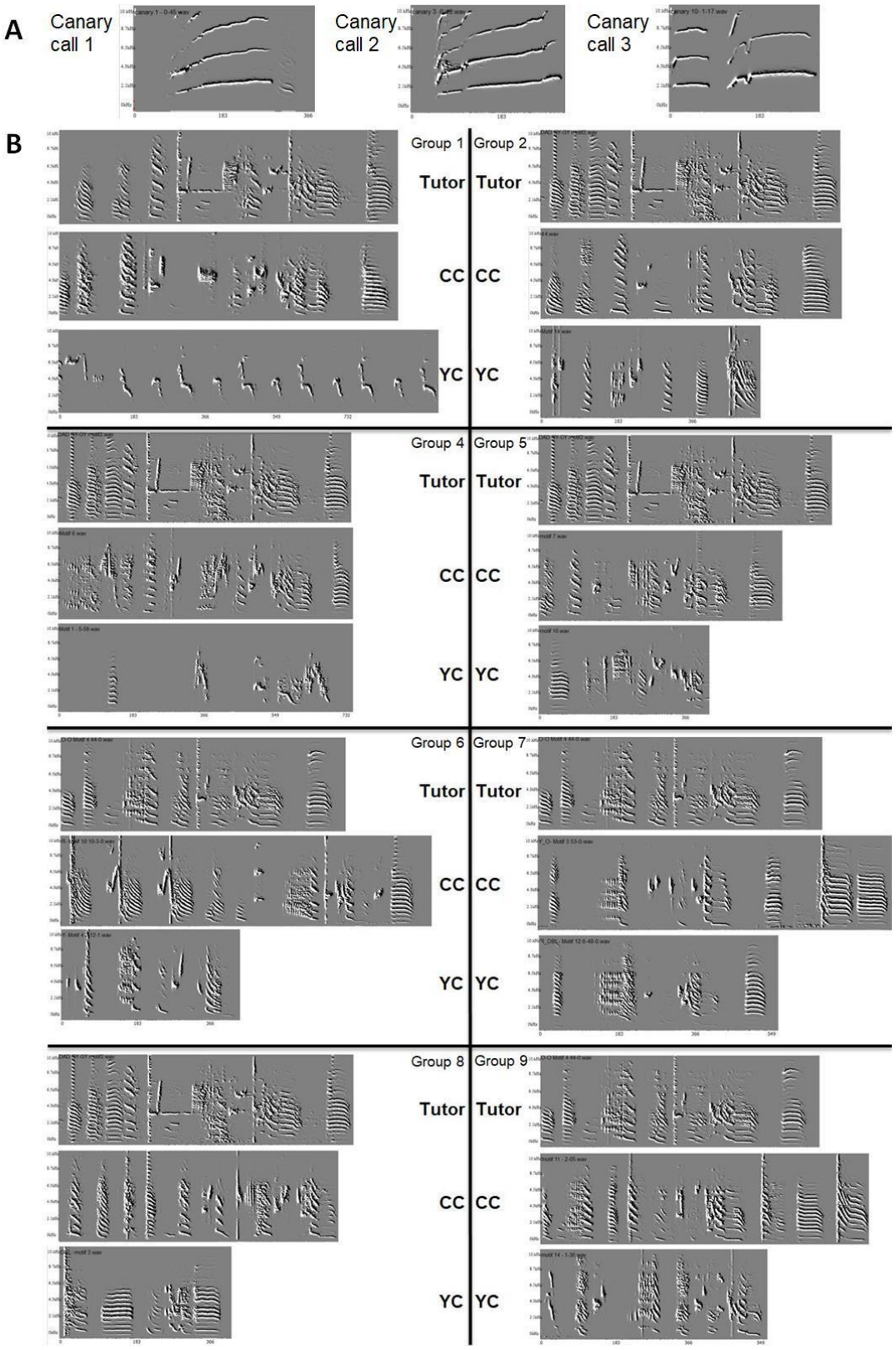


Figure 3.7. Spectrograms of exemplar canary calls and crystalized songs of subjects.

(A) Three exemplar calls recorded from canaries used as social partners for both CC and YC subjects. There were no significant differences in the acoustic similarity between any of the canary calls and the song syllables of CC and YC subjects (Table 3.2). (B) Spectrograms of crystalized songs of all tutor fathers and their paired CC and YC sons at 90 dph, with the exception of Group 3, which is shown in Figure 3.3A. Similarity scores for each group are shown in Figure 3.3C.

We used Sound Analysis Pro (Tchernichovski et al., 2000) to assess acoustic similarity between the canary calls and zebra finch song syllables. Sound Analysis Pro is commonly used to analyze vocalizations of various finch species, including zebra finches and canaries, and well-suited for comparing the harmonic stack acoustic structures typical of both species. We compared each of the three canary calls with five examples each of every syllable in the songs of each zebra finch subject in both contingent and yoked groups. We then performed paired-sample t-tests between the contingent and yoked groups on each of sixteen acoustic similarity analyses (Table 3.2). These included, for each individual canary call and all three averaged, the similarity score between all zebra finch syllables, the similarity score of the single most similar syllable, the average similarity of syllables in each song, and the average similarity of all syllables with non-zero similarity scores (to eliminate the possibility that differences might be driven or washed out by the high number syllables with similarities of zero). We found no significant difference between contingent and yoked subjects on any of these measures. Thus yoked birds were no more likely to copy canary calls than were their contingent brothers, and any copying that did occur happened at a very low fidelity (Figure 3.7B).

Table 3.2. Summary table of paired t-tests results of percent acoustic similarity between Contingent Condition and Yoked Control zebra finch subject song syllables and female canary social companion calls.
The first column indicates the aspect of zebra finch syllable similarity being analyzed, while the second indicates the canary call to which it is being compared. Standard deviations are shown in parentheses.

Zebra finch	Canary call	CC Mean	YC Mean	t value	Sig.
Average similarity score across all syllables	Call #1	2.712% (.947)	3.063% (3.318)	-.281	.786
Average similarity score across all syllables	Call #2	.072% (.110)	1.644% (2.759)	-1.721	.124
Average similarity score across all syllables	Call #3	.228% (.393)	2.036% (3.478)	-1.541	.162
Average similarity score across all syllables	Average of all calls	1.004% (.397)	2.248% (3.125)	-1.135	.289
Similarity score of the single most similar syllable	Call #1	12.556% (2.603)	16.889% (9.006)	-1.906	.093
Similarity score of the single most similar syllable	Call #2	2.667% (4.031)	10.000% (11.258)	-1.990	.082
Similarity score of the single most similar syllable	Call #3	4.556% (4.503)	9.222% (12.488)	-1.214	.259
Similarity score of the single most similar syllable	Average of all calls	6.593% (2.655)	12.037% (10.515)	-1.757	.117
Average similarity of syllables within each song	Call #1	2.728% (.940)	3.041% (3.312)	-.251	.808

Zebra finch	Canary call	CC Mean	YC Mean	t value	Sig.
Average similarity of syllables within each song	Call #2	.070% (.107)	1.642% (2.841)	-1.676	.132
Average similarity of syllables within each song	Call #3	.223 (.392)	1.947 (3.433)	-1.491	.174
Average similarity of syllables within each song	Average of all calls	1.007 (.398)	2.210 (3.143)	-1.906	.306
Average similarity of all syllables with non-zero similarity scores	Call #1	10.704 (.798)	13.227 (5.594)	-1.554	.159
Average similarity of all syllables with non-zero similarity scores	Call #2	2.667 (4.031)	7.818 (7.922)	-1.906	.093
Average similarity of all syllables with non-zero similarity scores	Call #3	4.333 (4.243)	7.094 (9.606)	-.932	.379
Average similarity of all syllables with non-zero similarity scores	Average of all calls	5.901 (2.301)	9.379 (7.313)	-1.571	.155

Video Stimulus

The female fluff-up stimulus shown to subjects lasted 0.8 seconds, consisting of the female erecting her feathers over 0.6 seconds and performing a 0.2 second shake of her body. The stimulus fluff-up is highly typical of female fluff-up behaviors in response to male song, which range from 0.37 – 1.12 seconds in duration, but are

most commonly 0.71 – 0.94 seconds including a 0.15 – 0.33 second shake, based on a sample of 500 fluff-ups from 8 adult females in our colony. Recording was conducted in a cage with a Lexan front and black felt backdrop to reduce glare, and lit with two daylight-balanced lights with aluminum reflectors. Two females were present in the cage simultaneously, as female finches often freeze in place when isolated from conspecifics. The second female was cropped from the video to create the final stimulus. A flight cage with five unpaired adult male zebra finches was placed behind the camera to draw female attention forward and elicit arousal behaviors. The camera was a Canon HD VIXIA HFM31, 3.3 megapixels resolution, filming in 1920 x 1080 resolution. Video playback to subjects was accompanied by background audio of female contact calling to provide a cue to birds facing away from the monitor that playback was occurring. If CC birds did not sing for the first ten minutes of a session, a 'reminder' playback was triggered every five minutes until the first instance of song, at which point no more reminder videos would be triggered.

Behavior Recording and Analysis

All subjects were recorded for the duration of every recording session, for one hour a day for 25 days. To obtain a detailed longitudinal cross-section of behavior over time, videos from every 5^{th} trial (trials 5, 10, 15, 20, and 25) were coded for each bird. Videos were coded for onset and offset of singing and video playback, and the arousal and motivation behaviors of fluff-ups, beak wipes, and landings on and departures from the perch nearest to the video monitor (Vyas et al., 2009). Behavioral coding was conducted using ELAN Linguistic Annotation software (Wittenburg et al.,

2006). All videos were independently coded by two research assistants, and any disparities in coding resolved by a third coder. Videos were first visually coded for arousal behaviors with the sound turned off to ensure coders were blind to experimental condition, before being coded for song and video playbacks.

Acoustic Recording and Analysis

At 90 dph, all subjects were video- and audio-recorded in the acoustic attenuation chambers in the presence of an unfamiliar female zebra finch to obtain directed song recordings. Subjects were taped for as long as required to obtain 20 high-quality recordings of motifs not occluded by background noise or female calls. The motifs used for analysis were therefore the first 20 unoccluded songs produced by each subject following song crystallization, to avoid any differential selection of motif across conditions. Recordings of genetic fathers of the subject were obtained using the same method, though these tutors were >150 dph at the time of recording. Each motif from each subject was compared to that of their biological and social father using the Similarity module of Sound Analysis Pro 2011 (Tchenichovski et al., 2000). The experimenter performing the acoustic analysis was blind to the condition of the bird from which each motif was obtained. Similarity scores generated by SAP2011 have three major components: the percent similarity score is computed over longer intervals (typically 50 - 70 msec) and reflects the amount of song material included from the tutor's song in the juvenile's motif. Accuracy is computed across shorter time windows (5 - 10 msec) and indicates how well the juvenile's song matches the tutor's song across these shorter segments. Sequential match incorporates the temporal order

(syntax) of the component syllables, and refers to the similarity of temporal order of final sections (as defined in the SAP 2011 manual) between the reference (tutor) song and the second (juvenile) sound. Overall similarity score is a calculated average of these three similarity components. For analysis, similarity results for all motifs were averaged for each subject into a single score for each similarity component score.

Quantification and Statistical Analysis

All statistical analyses were performed in IBM SPSS Statistics Software, version 22. Unless otherwise stated, all analyses were two-tailed, significance level was $p < 0.05$, and $n = 9$ subjects per group (18 birds total). In total, 20 males completed the experiment, but one sibling pair had to be excluded from analysis due to an equipment failure. A formal sample size calculation could not be conducted prior to experimentation, as the preliminary data necessary to perform such a calculation did not exist prior to this study. We chose to analyze 9 Contingent and 9 Yoked Control subjects, as 4-12 subjects per group is typical in animal studies of learning and performance. For each sibling pair, which brother was placed in the CC condition and which in the YC condition was determined at random by flipping a coin.

Our primary test for learning was based on the acoustic similarity between the subjects and their song tutor, which was also their social and genetic father. Because we used a paired-subjects design, with each Contingent subject paired with an age-matched genetic Yoked brother, we first performed paired subjects t-tests between the Contingent and Yoked groups on overall similarity, percent similarity, accuracy, and sequential match data obtained from Sound Analysis Pro. Significant results from the

t-test on percent similarity can be seen in Figure 3.3B and overall similarity in Figure 3.6B. We then performed a Wilcoxon Signed Ranks test to determine if, within these brother pairs, the Contingent subject was significantly more likely to develop superior song than its paired Yoked subject (see Figure 3.3C).

To determine which spectral features of the song contributed to differences in similarity, and whether Contingent and Yoked birds vary according to particular aspects of song structure, we first used paired-samples t-tests to compare mean values of average whole-song pitch, frequency modulation, entropy, goodness of pitch, and amplitude modulation. The means did not vary between groups (all ps > .05) (Table 3.1). We compared the distributions of each spectral component across groups using a Kolmogorov-Smirnov test. Only entropy differed between groups. YC subjects had a significantly broader distribution of entropy than CC subjects ($z = 1.414$, $p = .037$).

We then performed a principle components analysis constructed from the mean values of the same five primary spectral components (Figure 3.4A). All factors were normalized using Kaiser normalization and orthogonally rotated with Varimax. The groups differed significantly on PC2, which was weighted primarily by entropy, amplitude modulation and pitch (Figure 3.4B).

Using behavioral data acquired from video coding in ELAN, we performed linear regression analyses to determine the correlation between number of arousal behaviors (fluff-ups and beak wipes) performed by subjects across development and song learning outcomes, both within group and pooled across groups. We found a positive trend for CC birds between arousal behaviors and crystallized song similarity (Figure 3.6A). To determine if the groups differed in arousal behaviors at different

time-points in development, we then conducted a 2 (Condition: CC, YC) x 5 (Age: 40, 45, 50, 55, 60dph) repeated measures ANOVA on mean number of arousal behaviors (Figure 3.5). To determine whether our results were driven by differences in motivation to sing, leading to CC birds practicing song more than YC birds, we used video and audio recordings across development, sampled every five days from 35-60 dph, to count total number of song bouts per hour and total time spent singing for each subject. Using paired t-tests, we found no significant differences between CC and YC groups (Figure 3.8A). We then performed 2 (Condition: CC, YC) x 5 (Age: 40, 45, 50, 55, 60dph) repeated measures ANOVAs on the total duration of time spent singing each session (in seconds) and the number of song bouts per session. We found no significant main effect of Condition and no significant interaction effect. We found a significant effect of Age on song duration, $F(4,32) = 3.32$, $p = 0.022$. A Tukey HSD post-hoc test revealed a significant difference in song duration only between 40-50dph ($p < .05$). We also found a significant effect of Age on number of songs $F(2,32) = 3.23$, $p = 0.025$. A Tukey HSD post-hoc test revealed a significant difference in number of songs only between 40-50dph ($p < .05$) (Figures 3.8B and 3.8C).

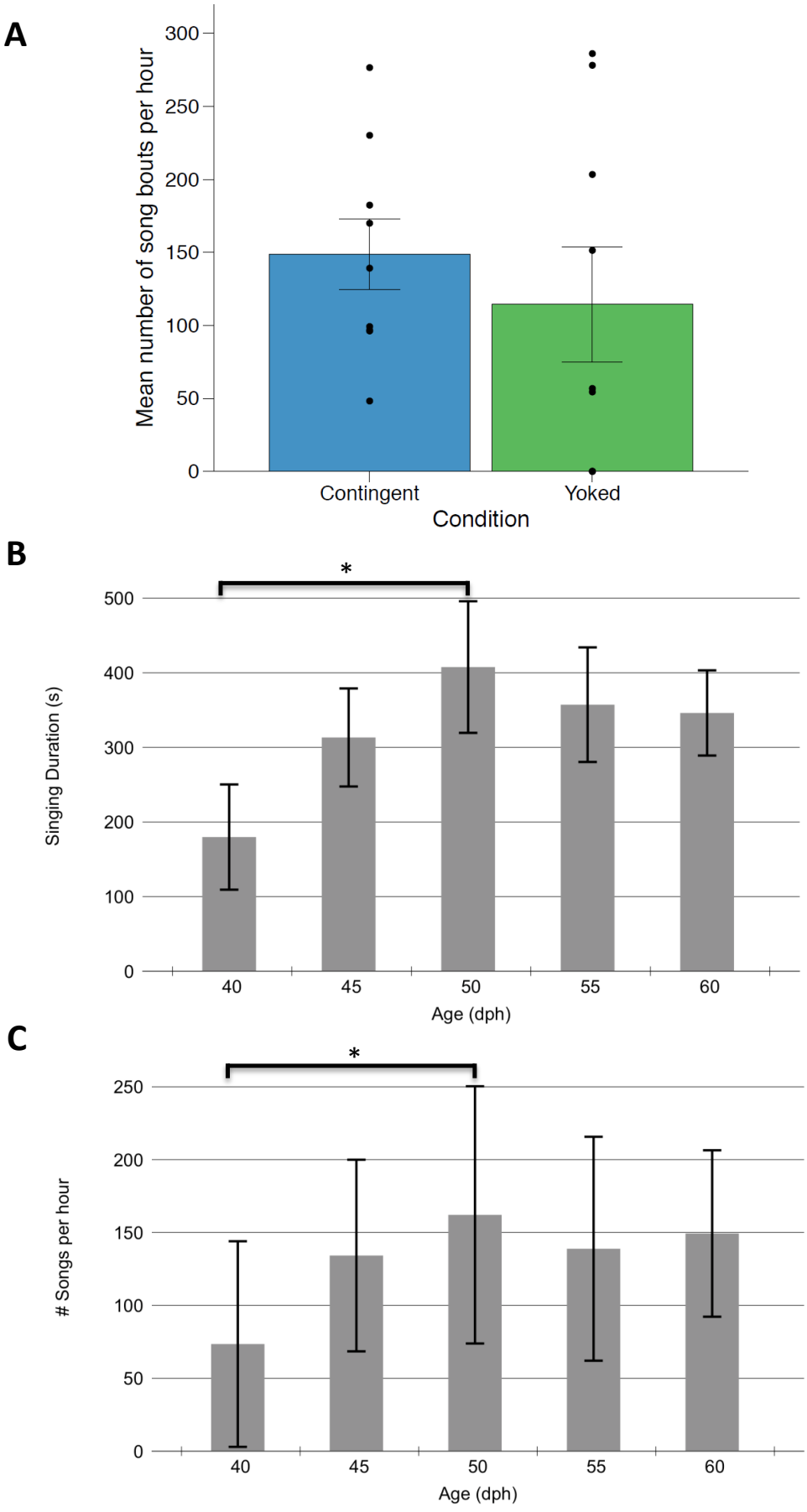

A
Mean number of song bouts per hour
300
250
200
150
100
50
0
Contingent
Yoked
Condition
B
*
Singing Duration (s)
500
400
300
200
100
0
40
45
50
55
60
Age (dph)
C
*
Songs per hour
250
200
150
100
50
0
40
45
50
55
60
Age (dph)

Figure 3.8. Rates of singing across groups and across development.
(A) Number of song bouts per hour produced by CC and YC subjects, averaged across developmental time, by group. No significant difference exists in singing rate ($t(8) = 0.682$, $p = 0.515$). Error bars ± 1 S.E. (B) We conducted a 2 (Condition: CC, YC) x 5 (Age: 40, 45, 50, 55, 60dph) repeated measures ANOVA on the total duration of time spent singing each session (in seconds). We found no significant main effect of Condition and no significant interaction effect. We found a significant effect of Age for duration, $F(4,32) = 3.32$, $p = 0.022$), A Tukey HSD post-hoc test revealed a significant difference in song duration only between 40-50dph ($p < .05$). Error bars ± 1 S.E. (C) We conducted a 2 (Condition: CC, YC) x 5 (Age: 40, 45, 50, 55, 60dph) repeated measures ANOVA on the number of song bouts produced per session. We found no significant main effect of Condition and no significant interaction effect. We found a significant effect of Age for song count, $F(2,32) = 3.23$, $p = 0.025$. A Tukey HSD post-hoc test revealed a significant difference in number of songs per hour only between 40-50dph ($p < .05$). Error bars ± 1 S.E.

Data and Software Availability

All behavioral and learning data generated and analyzed during this study are publicly available on Mendeley Data at http://dx.doi.org/10.17632/63db6zc5fm.2. The custom-written Python code used in this study to extract behavioral measures from ELAN video coding software into a spreadsheet format and calculate contingencies between behaviors is available upon request.

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REFERENCES

Adret, P. (2003). Vocal imitation in blindfolded zebra finches (*Taeniopygia guttata*) is facilitated in the presence of non-singing females. *Journal of Ethology, 22*, 29-35.

Albert, R., Schwade, J.A., & Goldstein, M.H. (2017). The social functions of babbling: Acoustic and contextual characteristics that facilitate maternal responsiveness. *Developmental Science, e12641*.

Baran, N.M., Peck, S.C., Kim, T.H., Goldstein, M.H., & Adkins-Regan, E. (2017). Early life manipulations of vasopressin-family peptides alter vocal learning. *Proceedings of the National Society B, 284*, 20171114.

Borland, J.M., Frantz, K.J., Aiani, L.M., Grantham, K.N., & Albers, H.E. (2017). A novel operant task to assess social reward and motivation in rodents. J. *Neuroscience Methods, 287*, 80-88.

Brenowitz, E.A., & Beecher, M.D. (2005). Song learning in birds: diversity and plasticity, opportunities and challenges. *Trends in Neuroscience, 28*, 127-132.

Chen, Y., Matheson, L.E., & Sakata, J.T. (2016). Mechanisms underlying the social enhancement of vocal learning in songbirds. *Proceedings of the National Academy of Science*, *113*, 6641-6646.

Derégnaucourt, S., Poirier, C., Kant, A.V., Linden, A.V., & Gahr, M. (2013). Comparisons of different methods to train a young zebra finch (*Taeniopygia guttata*) to learn a song. *Journal of Physiology – Paris, 107*, 210-218.

Eales, L.A. (1989). The influences of visual and vocal interaction on song learning in zebra finches. *Animal Behaviour, 37*, 507-508.

Gadagkar, V., Puzerey, P.A., Chen, R., Baird-Daniel, E., Farhang, A.R., & Goldberg, J.E. (2016). Dopamine neurons encode performance error in singing birds. *Science, 354*, 1278-1282.

Goldstein, M.H., King, A.P., & West, M.J. (2003). Social interaction shapes babbling: Testing parallels between birdsong and speech. *Proceedings of the National Academy of Science, 100*(13), 8030-8035.

Goldstein, M.H., & Schwade, J.A. (2008). Social feedback to infants' babbling facilitates rapid phonological learning. Psychological Science, *19*, 515-522.

Guillette, L.M., & Healy, S.D. (2017). The roles of vocal and visual interactions in social learning zebra finches: A video playback experiment. *Behavioral Processes*, *139*, 43-49.

Gultekin, Y.B., & Hage, S.R. (2018). Limiting parental interaction during vocal development affects acoustic call structure in marmoset monkeys. *Science Advances*, *4*, eaar2012.

Heimovics, S.A., Cornil, C.A., Ball, G.F., & Riters, L.V. (2009). D1-like dopamine receptor density in nuclei involved in social behavior correlates with song in a context-dependent fashion in male European starlings. *Neuroscience*, *159*, 962–973.

Ikebuchi, M., & Okanoya, K. (1999). Male zebra finches and Bengalese finches emit directed song to the video images of conspecific females projected onto a TFT display. *Zoological Science*, *16*, 63-70.

Immelmann, K. (1969). Song development in the zebra finch and other estrildid finches. In *Bird Vocalizations*, R.A. Hinde ed. (Cambridge: Cambridge University Press), pp. 61-81.

Kuhl, P. (2003). Human speech and birdsong: Communication and the social brain. *Proceedings of the National Academy of Science, 100*(17), 9645-9646.

Kuhl, P. (2006). Is speech learning 'gated' by the social brain? *Developmental Science, 10*, 110-120.

Marler, P. (1970). A comparative approach to vocal learning: Song development in white-crowned sparrows. *Journal of Comparative and Physiological Psychology, 71*, 1-25.

Marler, P. (1997). Three models of song learning: Evidence from behavior. J. *Neurobiology, 33*, 501–516.

Marler, P., & Nelson, D.A. (1992). Action-based learning: a new form of developmental plasticity in birdsong. *Netherlands Journal of Zoology, 43*, 93-103.

Martin, L., & Iceberg, E. (2015). Quantifying social motivation in mice using operant

conditioning. *Journal of Visualized Experiments, 102*, 53009.

Morrison, R.G., & Nottebohm, F. (1992). Role of a telencephalic nucleus in the delayed song learning of socially isolated zebra finches. *Developmental Neurobiology, 24*, 1045-1064.

Newman, S.W. (1999). The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. *Annals of the New York Academy of Sciences, 877*, 242-257.

O'Connell, L.A., & Hofmann, H.A. (2011). The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *Journal of Comparative Neurology, 519*(18), 3599-3639.

Panksepp, J., & Moskal, J. (2008). Dopamine and seeking: Subcortical reward systems and appetitive urges. In *Handbook of Approach and Avoidance Motivation*, A.J. Elliot ed. (New York: Taylor and Francis), pp. 67-88.

Perez, E.C., Fernandez, M.S.A., Griffith, S.C., Vignal, C., & Soula, H.A. (2005). Impact of visual contact on vocal interaction dynamics of pair-bonded birds. *Animal Behaviour, 107*, 125-137.

Petrinovich, L. (1972). Psychobiological mechanisms in language development. In *Advances in Psychobiology,* G. Newton, and A.H. Riesen, eds. (Wiley, New York), Vol. 1.

Rauceo, S., Harding, C.F., Maldonado, A., Gaysinkaya, L., Tulloch, I., & Rodriguez, E. (2007). Dopaminergic modulation of reproductive behavior and activity in male zebra finches. *Behavioral and Brain Research, 187*, 133-39.

Sasaki, A., Sotnikova, T.D., Gainetdinov, R.R., & Jarvis, E.D. (2006). Social context-dependent singing-regulated dopamine. *Journal of Neuroscience, 26*, 9010–9014.

Syal, S., & Finlay, B.L. (2011). Thinking outside the cortex: social motivation in the evolution and development of language. *Developmental Science, 14*(2), 417-430.

Takahashi, D.Y., Liao, D.A., & Ghazanfar, A.A. (2017). Vocal learning via social reinforcement by infant marmoset monkeys. *Current Biology*, *27*, 1844-52.

Tchernichovski, O., Lints, T., Mitra, P.P., & Nottebohm, F. (1999). Vocal imitation in

zebra finches is inversely related to model abundance. *Proceedings of the National Academy of Science, 96*, 12901-12904.

Tchernichovski, O., Nottebohm, F., Ho, C.E., Bijan, P., & Mitra, P.P. (2000). A procedure for an automated measurement of song similarity. *Animal Behavior, 59*, 1167-1176.

Theofanopoulou, C., Boeckx, C., & Jarvis, E.D. (2017). A hypothesis on the role of oxytocin in the social mechanisms of speech and vocal learning. *Proceedings of the National Society B, 284*, 20170988.

West, M.J., & King, A.P. (1988). Female displays affect the development of male song in the cowbird. *Nature, 334*, 244-246.

Williams, H. (2004). Birdsong and singing behavior. *Annals of the New York Academy of Sciences, 1016*, 1-30.

Wittenburg, P., Brugman, H., Russel, A., Klassmann, A., & Sloetjes, H. (2006). ELAN: a professional framework for multimodality research. In *Proceedings of LREC, Fifth International Conference on Language Resources and Evaluation*.

Vyas, A., Harding, C., Borg, L., & Bogdan, D. (2009). Acoustic characteristics, early experience, and endocrine status interact to modulate female zebra finches' behavioral responses to songs. *Hormones and Behavior, 55*, 50-59.

Zann, R.A. (1996). *The Zebra Finch: A Synthesis of Field and Laboratory Studies* (Oxford University Press, Oxford).

CHAPTER 4

CONTINGENT PARENTAL RESPONSES GUIDE ZEBRA FINCH SONG LEARNING IN NATURALISTIC SOCIAL CONDITIONS

Abstract

Accurate vocal learning in zebra finches was traditionally thought to be the product of memorization and imitation of a song model (e.g. Roberts et al., 2012; Deshpande, Pirlepesov & Lints, 2014; Tchernichovski et al., 2001). Recent work, however, has, demonstrated the potency of social stimuli for enhancing song learning (Chen, Matheson, & Sakata, 2016; Derégnaucourt et al., 2013; Eales, 1989). Zebra finches also show improved learning in the presence of non-singing female conspecifics (Williams, 2004; Adret, 2003). What mechanisms drive the superior learning associated with live social interactions? We recently found that 'fluff-ups', a non-vocal female arousal cue, facilitated song learning when played over video contingently on juvenile song production throughout development (Carouso-Peck & Goldstein, 2019a). However, it is not known whether adults naturally respond in a contingent fashion to immature song, and whether young finches incorporate naturally-occurring feedback into song learning. To investigate whether social influences affect song learning, the present study addressed the nature and significance of maternal and paternal social cues associated with the development of song. We recorded unmanipulated zebra finch families throughout juvenile development and analyzed their social interactions. We found that behaviors of both parents affected

offspring song outcomes. Juvenile song structure learning outcomes could be predicted by the amount of contingent ‘fluff-up’ behaviors they received from their mothers throughout development. Fathers often sang immediately after a juvenile had sung, and we found that contingent paternal singing predicted better song learning in their sons. Conversely, when fathers sang before the juvenile sang, juveniles learned the father’s song less accurately. Our data suggest that parental vocal and gestural feedback plays an instructive role for song learning in zebra finches.

Introduction

Birdsong is the most-studied model system for human speech acquisition due to the behavioral, neural, and genetic similarities between the two learning processes (Bolhuis, Okanoya, & Scharff, 2010; Doupe & Kuhl, 1999; Goldstein, King & West, 2003). One similarity which has received comparatively little attention is dependence on the social environment: young birds and babies need to interact with adults to develop vocalizations with proper form and function (Doupe & Kuhl, 1999; Goldstein & Schwade, 2010; Smith, King & West, 2000). In the most commonly studied songbird, the zebra finch, juveniles pay more attention to and learn better from tutors with which they can socially interact, resulting in improved song learning outcomes (Chen, Matheson & Sakata, 2016). We recently found evidence that cues from non-singing females can also guide juvenile song development: adult female 'fluff-ups' – an arousal behavior – presented contingently via video on the immature plastic song of juveniles throughout development facilitated song learning (Carouso-Peck & Goldstein, 2019a). However, it remains unknown to what extent females naturally exhibit arousal cues, use those cues to respond to juvenile songs, or whether maternal feedback guides song development in a naturalistic social context. It is also unknown whether contingent paternal reactions, such as contingent song production, influence juvenile song learning outcomes.

Much of what we understand about the process of song learning in birds comes from classic work on sparrows. Sparrows are highly territorial, and use their song to defend their territory (e.g. Nowicki, Searcy & Hughes, 1998; Akçay et al., 2013; Marler & Peters, 1982), unlike many songbird species which use their song

exclusively for attracting a mate. The use of sparrow song in aggressive interactions makes direct social interaction with adult tutors during the song learning process a dangerous prospect for juvenile learners. This has resulted in an 'eavesdropping' learning system in which young sparrows first memorize adult song from a safe distance during an initial 'sensory' stage, and then practice imitating the song during a temporally distinct 'sensorimotor' stage (Beecher et al., 2007; Konishi, 1965; Nelson & Marler, 1994; Soha & Marler, 2001; Marler & Peters, 1982). The young do not require social feedback to learn. This model for learning presumes a passive role of the juveniles, because mere exposure to a taped tutor song can lead to a good imitation (Hultsch & Todt, 1992). This system has been well demonstrated in sparrows, but has often been overgeneralized into an assumption that all songbirds learn in a similar imitative fashion, with social influences playing at most a minor role (see Carouso-Peck & Goldstein, 2018).

When non-imitative social guidance of song structure was discovered, it was in another atypical songbird: the brood-parasitic brown-headed cowbird. Non-singing females were found to respond selectively to juvenile male song elements with rapid lateral wing movements ('wing strokes'), that the juveniles use to guide their song development (West & King, 1988). These rapid movements were only perceptible to humans when video records of the females were and played back at a fraction of real speed. Such social influences on early development, termed *socially guided vocal learning* (West & King, 1985), have not been directly studied in bird species other than the cowbird. Social interactions organized around the immature vocalizations of juveniles have the potential to play an active role during development in social species

more broadly, but have rarely been investigated.

However, there have long been hints that social influences affect song learning in the zebra finch. For instance, their song is better learned from live tutors than from tapes or non-interactive playback paradigms (Chen, Matheson, & Sakata, 2016; Deregnaucourt et al., 2013; Eales, 1989). The juveniles also play an active role in selecting their tutor, and this choice is primarily influenced by social interactions, suggesting a learning mechanism beyond mere exposure. The salience of adult tutor song is based on the amount of parental care delivered to fledglings (Williams, 1990), physical proximity (Mann & Slater, 1995), aggression towards the pupil (Clayton, 1987; Jones & Slater, 1996), his mating status and partner (Eales, 1987; Mann & Slater, 1994), visual cues such as color morph (Mann & Slater, 1995; Mann, Slater, Eales, & Richards, 1991), and auditory information such as song similarity between the father and subsequent song tutors (Clayton, 1987). Juvenile males prefer to learn to sing from their father both in the wild and in laboratory settings, even if other potential song models are available (Bohner, 1983; Zann, 1990), although they learn from multiple tutors depending on the amount of parental care (Williams, 1990). In addition, when multiple male siblings are raised together by an adult male, their song copy fidelity is highly variable, compared to consistently accurate song imitation when a father raises a single male juvenile (Tchernichovski & Nottebohm, 1998). Finally, the structure and timing of song development itself, with a high degree of overlap between the sensory and sensorimotor phases of song learning (Slater, Eales & Clayton, 1988; Roper & Zann, 2006), offers opportunities for social feedback to influence song learning, as it does in the cowbird.

Non-singing female listeners also affect zebra finch song development, as males raised with deaf females sing more frequently and develop more atypical songs than those raised with hearing females (Williams, 2004), and blindfolded males develop more accurate song when raised with a female sibling (Adret, 2003). However, the mechanisms underlying such enhanced learning are unknown. Enhanced learning during live interactions may be explained by feedback from parents contingent on a juvenile action, which may increase learning due to heightened arousal or attention (ten Cate, 1991). To date, the sole observational study investigating contingencies in interactions between zebra finch tutors and pupils in natural family settings did not find clear relations supporting that social behavior of the tutor reinforces vocal learning (Houx & ten Cate, 1998). However, this study examined behavior in real time, as perceived by a human observer, and not at the finer temporal scale at which birds are capable of perceiving motion (Healy et al., 2013; Carouso-Peck & Goldstein, 2019b).

Zebra finches are raised jointly by their parents well into the sensorimotor learning phase (Zann, 1996), presenting the opportunity for both paternal and maternal behaviors to influence song development. Discovering which maternal behaviors might be relevant to juvenile song learning requires determining which behaviors females naturally display in response to attractive male displays. Three types of female visual signals have been linked to mate preference in female songbirds: copulation-solicitation displays in numerous songbird species (Anderson, 2009; King & West, 1977; O'Loghlen & Beecher, 1997; Searcy & Marler, 1981; Vallet & Kreutzer, 1995), wing strokes in brown-headed cowbirds (West & King, 1988), and shakes or feather-

fluff-ups in the zebra finch (Vyas et al., 2009). Wing strokes and fluff-ups may be signals that females use to indicate song preference and thereby influence vocal development in their sons. Male tutors may similarly respond to juvenile song with contingent song to guide vocal development. To investigate whether social influences affect song learning in the zebra finch, the present study addresses the nature and significance of maternal and paternal social interactions associated with the development of juvenile song in unmanipulated families. We analyzed video recordings of naturalistic social interactions and annotated the timing of juvenile and adult male songs as well as female gestures, such as wing strokes and fluff-ups, occurring temporally close to songs of the juvenile males and their fathers, and investigated whether these interactions were correlated with juvenile song learning.

Methods

Breeding

Eight male-female pairs of adult zebra finches were selected from an outbred population maintained in the AALAC accredited animal facility in the Cornell University Psychology Department. Pairs were placed in single stainless steel cages (46 x 44 x 36 cm, n = 6 pairs, and 62 x 44 x 36 cm, n = 2 pairs) which contained wooden perches, a plastic nest box, and coconut nesting material, in a communal room that allowed visual and acoustic contact with each other and with birds in other aviaries. The birds were kept on a 14:10 light cycle with full-spectrum lighting. Housing rooms were maintained at 24º C and 50% relative humidity. Birds were

provided with mixed foreign finch seed, water, grit, and access to cuttle bone *ad libitum*. The birds received water for bathing and greens as a dietary supplement weekly. Following this study the animals were returned to the colony. All procedures were approved by Cornell's IACUC committee under protocol 1988-0135.

Each pair nested, laid eggs, hatched, and fledged 2-5 young (M = 3.88, SD = 1.25), which resulted in 14 juvenile males and 15 juvenile females total. Juveniles were color banded at approximately 3 weeks of age (M = 21, *Range* = 17 - 26 days; age always refers to the hatching date of the oldest juvenile). Families were transferred to larger cages (62 x 44 x 36 cm) when the oldest chick was 24 days post hatch (dph). Nest boxes were removed at about 35 days in order to prevent re-nesting of the parents during the study.

Behavioral recordings

Video recordings of family interactions began between 21 and 35 dph (M = 27, SD = 5.2) and continued at approximately 3-day intervals until 70 - 95 dph. In this study, we focused our analysis on videos recorded between 30 and 75 dph. Within this interval each family was recorded for about 16 hours (M = 16, SD = 2.46). For each session, the entire cage containing the parents and offspring was placed overnight in a sound attenuating enclosure (140 x 98 x 80 cm) lined with Sonex sound attenuating foam.

For each recording, family interactions were recorded for one hour the following morning using a Sennheiser K6/ME64 shotgun microphone connected to a Canon MiniDV ZR930 camcorder with Fujifilm DVCassette miniDVs. After each

recording, family cages were returned to the communal housing room until the subsequent recording. Families were kept together until about 99 days (*SD* = 9 days). Juveniles were then transferred to single-sex aviaries and housed with other juvenile birds (90 cm W, 122 H, 60 D, each containing up to 20 individuals).

Ethical note

All protocols were approved the Cornell Institutional Animal Care and Use Committee. Animal subject treatment complied with the standards defined by the Committee. The study was designed to minimize number of birds used, disturbance, and stress.

Behavioral coding and analysis

Sound files were created from video recordings using Soundtrack Pro (version 3.0.1) and uncompressed sound files were saved as wav files. ELAN Linguistic Annotator software (Wittenberg et al., 2006) was used to synchronize the video and sound files and to code the behavior of juvenile males and their parents. ELAN permits annotations of multiple categories of behavior from individual animals with a frame-by-frame accuracy onto different fields associated with the video recording.

Initial analyses examined dynamics of song productions between the fathers and juvenile males. Thus, all adult male and juvenile songs were first annotated with single frame accuracy. Song in zebra finches occurs in bouts, which we defined in behavioral annotations as singing without silent intervals greater than 1 second. Songs were coded as *directed* whenever the singer initiated singing while clearly facing

another individual, and was accompanied by courtship behavior such as a raised crest, side-to-side head movement, or courtship dance (Williams, 2001). All other songs were coded as *undirected*. Directed songs were most often performed by the adult male directed to the adult female. Throughout the observation period, juvenile males rarely directed their songs towards their mothers when their father had recently sung or was about to sing (M = 2, SD = 1.77 per juvenile), thus we used the total juvenile male song in subsequent analysis. Thus *directed* singing events in the subsequent analysis always refer to song by the adult male directed toward the adult female. Next, 15-second time windows before and after the songs were marked in ELAN for every adult and juvenile male. Some song bouts were produced in quick succession, leading to overlap between the after and before time windows. When these overlaps occurred, the time between songs was labeled as "between" (Figure 4.1).

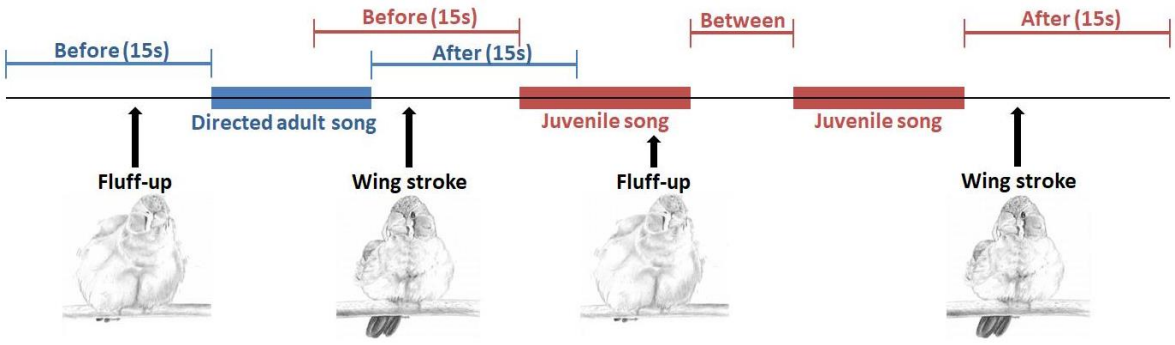

Figure 4.1. Example of temporal relationships between juvenile song and parental feedback.
After coding adult male (blue) and juvenile male (red) song bouts ('during' song periods), 15 second time windows were created before each song onset and after each song completion. When two juvenile songs followed in rapid succession of < 15 seconds, intervening time periods were labeled as 'between'. Female fluff-ups and wing strokes were coded only in these song-adjacent time windows. Contingent interactions were assessed based on the temporal relations between behaviors occurring within these windows (e.g. 'directed adult song after juvenile song', 'female fluff-up before juvenile song' etc.)

Because juvenile songs often occurred in quick succession within the 15-second time window, the possibility existed for several juvenile songs to be binned together, counting as only a single event before a contingent song from the father. Thus we analyzed these events in two ways. First, we counted the number of times that a paternal song followed within 15 seconds of a juvenile song or song bout (Figure 4.3A). Second, to account for repeated juvenile songs in a bout, we counted the number of individual juvenile songs that preceded a paternal song within 15 seconds (Figure 4.3B). However, when analyzing the contingency of juvenile songs after paternal songs, we noted that fathers never sang multiple times within the 15 second windows. Therefore, we analyzed these events only by counting the number of paternal songs that preceded a juvenile song within 15 seconds (Figure 4.4).

Wing strokes and fluff-ups by the mother were coded during song and in the fifteen seconds before and after songs. Wing strokes were defined as a single fast movement of one (unilateral) or both (bilateral) wings, made independent of large body movements (West & King, 1988). The tallies did not include wing movements made within 1 second of landing from flight, as birds tend to adjust their feathers immediately after landing. Wing strokes were coded after hopping and jumping but only after the female was in contact with the perch. Wing strokes were not coded during preening, or when the movement of the tail appeared to cause the movement of the wing. Tiny wing adjustments during sudden bending were not coded as wing strokes. As the movements can be brief and subtle, all wing strokes were independently coded by two individuals. A third person then checked both coders' annotations and resolved any discrepancies. Wing stroke annotations from this final

assessment were used in the data analysis. Across all families, there was an average agreement between the final assessment and the first coder of 61.3% (*SD* = 0.21), and with the second coder of 73.3% (*SD* = 0.19).

Fluff-ups are brief erections of the feathers accompanied with a quick shaking movement of the entire body from side to side followed by smoothing the feathers back down (Vyas et al., 2009). Initially, two independent coders annotated the presence of fluff-ups. As they were highly consistent over 30% of the data (inter-coder reliability: *M* = 0.87; *SD* = 0.24), annotations of only one coder were used in subsequent analyses.

Behavioral contingencies

From the annotations, we determined the occurrence of directed, undirected, and total (directed + undirected) adult male songs, juvenile songs, and female wing strokes and fluff-ups for the entire developmental period for each family. Juvenile song was rarely classified as directed (*M* = 2, *SD* = 1.77, per juvenile across the entire observation period), thus we used total juvenile song in all analyses. We also analyzed the total number of wing strokes by pooling uni- and bilateral wing strokes. Behaviors were assigned to categories based on their temporal relationship, namely how many behaviors occurred before, during, after, and between adult male or juvenile songs (Figure 4.1).

Mature song recordings

Mature song was recorded from sons (n = 11) following song crystallization

(Immelmann, 1969), in the fourth month after hatching (*M* = 104, *SD* = 9.59 days) and from their fathers (n = 8) (see Figure 4.2 for example spectrograms). Three sons did not sing during these initial recording sessions, so their songs were recorded in the fifth month after hatching, between 142-153 dph (total n = 14). Before all recordings, males were placed in a sound-proof room overnight in a 46 x 44 x 36 cm cage. The following morning, an adult female zebra finch was placed in an identical cage next to the male's cage and recording began. If the male did not sing within 60 minutes, further recordings were attempted on subsequent days until we obtained at least 10 songs. The mature songs were video and audio recorded, the video recordings were annotated in ELAN software to locate directed songs visually, and only directed songs were selected for subsequent song analyses.

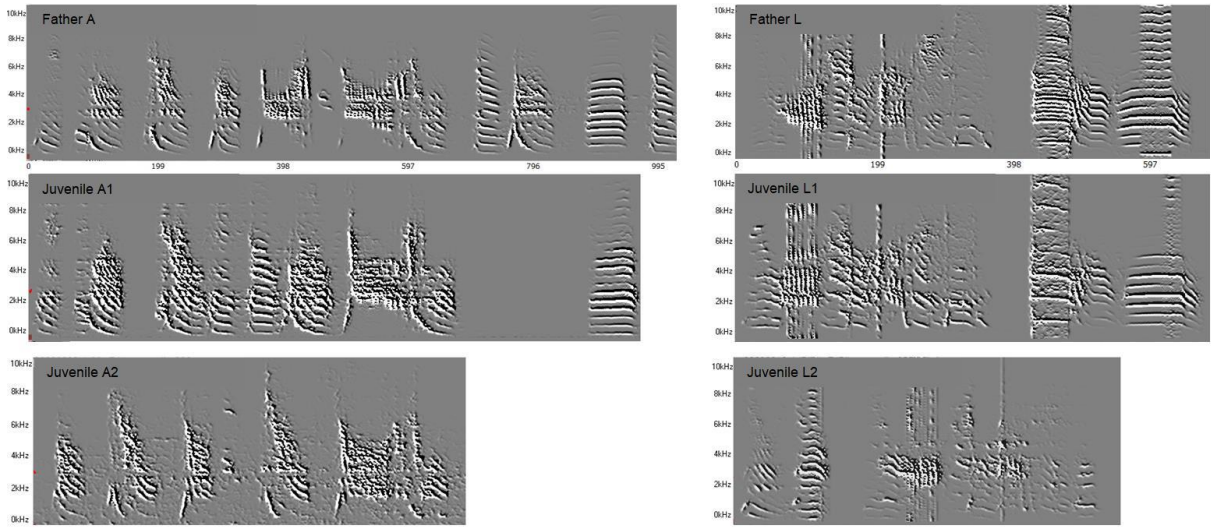

Figure 4.2. Comparison spectrograms examples of crystalized song outcomes.
Spectrograms from two families of subjects, Family A (left) and L (right). The songs of fathers (top row) were compared with the final songs of their sons (second and third rows) following juvenile song crystallization. Song outcomes varied considerably even within the same families, depending on rate, timing and form of parental responsiveness.

Analysis of mature song

Zebra finch song consists of a series of individually distinct acoustic elements, called syllables, which are sequentially organized into a consistent pattern, the song motif. The motif is initially preceded by a train of repetitive introductory elements. A single song bout contains between 1 and 8 motif repetitions (Price, 1979). Zebra finch song is often described as highly stereotyped. In actuality, males usually sing slightly different motif variants, containing different numbers of notes (Helekar et al., 2000; Scharff & Nottebohm, 1991; Sturdy, Phillmore, & Weisman, 1999; Menyhart et al., 2015). The most common motif occurs in about 60% of song bouts and is termed the "dominant" or "canonical" motif (Scharff & Nottebohm, 1991; Zann, 1996). For the purpose of acoustic analysis, we randomly choose 10 renditions of the dominant motif from each recording (both sons and fathers) from samples that did not contain background noise, female calls or cage noise. We also excluded the first motif of each song bout as it tends not to have the consistency of subsequent motifs. To assess song learning, we then compared 10 motifs from the mature song of each son to 10 motifs from their respective fathers, performing 10 x 10 comparisons within the similarity batch feature of Sound Analysis Pro 2A.04 (SAP2) software (Tchernichovski et al., 2000; Tchernichovski, Swigger & Partha, 2004). For our analyses, we used the default settings of the similarity module and derived the three different measurements of similarity between songs that this software package provides:

Percent similarity attempts to capture similarity in overall feature values between two songs and is computed over 50 - 70 msec intervals of sound. *Accuracy* is computed across shorter time windows (5 - 10 msec) and indicates the accuracy of the

vocal match between the two songs across similar segments. *Sequential match* accounts for the temporal order (syntax) of sounds. Sequential match is calculated by sorting superior similarity sections according to their temporal order in reference to sound 1, and then examining their corresponding order in sound 2. Superior similarity sections are the final results of a filtering process that omits sections that explain very little similarity and are likely to be noise.

Statistical analysis of song outcome measures

To assess the contribution of behavioral interactions during development on song learning, we correlated behaviors occurring in the 15 second time windows with similarity scores obtained between the songs of each father and the mature songs of their sons. To control for effects of juvenile song activity and the number of observations per family, we controlled for amount of singing by partialling out juvenile song rate per hour from the correlations.

Some families contained more than one son, and so the 14 juvenile males could not be treated as independent data points. To analyze the effects of family on the three song outcome measures, we conducted separate intercept-only linear mixed models with family as the random factor and song similarity measures as the dependent variable in each analysis. For the three measures, 4.7% of variance in accuracy, 24% of variance in similarity, and 73% of variance in sequential match was attributed to family. Because family accounted for a large proportion of variance on the latter two outcome measures, family identity was controlled for in all subsequent data analyses involving learning measures.

We performed a series of principal component analyses to examine the joint and separate contributions of paternal and maternal contingent responses to juvenile song learning outcomes. All factors were normalized using Kaiser normalization and orthogonally rotated with Varimax. The initial analysis included all paternal singing behaviors, maternal feedback behaviors (wing strokes and fluff-ups) and juvenile singing behaviors. In order to determine which aspects of these components were most strongly correlated with which outcome measures, we then ran PCAs on each set of behaviors separately: one for paternal singing in relation of juvenile song, one on maternal behaviors in relation to juvenile song, and one on juvenile song production in relation to adult song. We then performed partial correlations, controlling for family, between each resulting factor with an Eigenvalue > 1 and the three song outcome measures of percent similarity, accuracy, and sequential match.

Results

Parental responses to juvenile song

Adult males responded contingently to their juvenile's song. They sang more songs after their son sang (M = 14.5, SD = 9.99) than before (M = 6.21, SD = 4.08) ($t(13) = -3.832$, $p = .002$), and juvenile males sang more songs before their fathers started to sing (M = 26.71, SD = 17.93) than after (M = 19.93, SD = 12.36) ($t(13) = 2.519$, $p = .026$). We determined the percentage of juvenile vocalizations that elicited adult male song by calculating the percentage of juvenile song occurring immediately before the father's compared to the total juvenile song produced. This varied widely across juveniles (M = 4.26%, SD = 2.86, *Range* = 0.44 - 9.12 %). Females responded

with wing strokes during an average of 10.8% of songs (*SD* = 0.050), and displayed wing strokes more frequently during the song of their mate (*M* = 13.4%, *SD* = 5.1) than the subsong and plastic song of their sons (*M* = 9.4%, *SD* = 4.4), ($t(13) = -2.217$, $p = 0.045$). While the majority of songs did not receive wing strokes, those that did often elicited more than one, with an average of 2.08 wing strokes per adult song (*SD* = 0.525, *Range* = 1-19) and 1.07 wing strokes per juvenile song (*SD* = 0.365, *Range* = 1-10) within 5 seconds of singing. Fluff-ups were rare compared to wing strokes. Mothers responded with fluff-ups during only 1.7% of songs (*SD* = 0.016), and only 2.6% of songs received a fluff-up within 15 seconds of singing (*SD* = 0.021). The rate of maternal fluff-ups during adult male song (*M* = 2.6% of songs, *SD* = 0.021) was twice that elicited by juvenile songs (*M* = 1.3% of songs, *SD* = 0.012) ($t(13) = -2.558$, $p = 0.024$). Rates of behaviors across recordings, per hour, and per juvenile song within 15 seconds of singing are summarized in Table 1.

Table 4.1. Behavioral activity of mothers, fathers, and juveniles across development. Described as mean number of behaviors per bird over the entire recording period, and mean number of behaviors per hour, and mean proportion of juvenile songs which received a response during singing or within 15 seconds after.

	Activity over recording period		**Activity per hour**		**Percentage of juvenile songs receiving response**	
	Mean ± SD	Min - Max	Mean ± SD	Min - Max	Mean ± SD	Min - Max
Juvenile song activity	1147.07 ± 659.61	358 - 2682	72.2 ± 50.39	24 - 216	-	-
Adult male song activity	456.63 ± 283.5	251 - 1042	30.32 ± 23.58	13 - 84	4.57 ± 2.85%	0.33 - 10.19%
Adult female wing strokes	1716.88 ± 996.49	614 - 3287	108.05 ± 65.44	46 - 228	11.31 ± 5.15%	3.36 – 21.9%
Adult female fluff-ups	139.5 ± 94.87	54 - 306	8.5 ± 5.28	4 - 17	1.94 ± 1.57%	0.56 – 4.67%

Effects of social feedback on song learning

Juveniles copied their father's song well, as expected, but with considerable variation between individuals in terms of percent similarity (M = 73.06, SD = 11.16, *Range* = 49.44 – 95.34), accuracy (M = 75.3, SD = 2.09, *Range* = 71.4 – 79.11), and sequential match (M = 59.41, SD = 14.53, *Range* = 42.24 – 91.53). To determine whether this variation in song learning was linked to social interactions between sons and both their fathers and their mothers, we correlated behavioral contingencies occurring in the 15-second time windows surrounding song production with the three measures of final song similarity obtained by comparing the mature song of the son to the song of his father, and found significant effects on sequential match. The incidence of directed songs by the father just *after* the juvenile male sang was significantly

positively correlated with the eventual sequential match to the father's song (r(10) = 0.734, p = 0.007) (Figure 4.3A). The number of juvenile songs *before* directed adult male song was also significantly positively correlated with final sequential match between juvenile and tutor (r(10) = 0.775, p = 0.003) (Figure 4.3B). In contrast, more frequent juvenile song *after* father song was significantly negatively correlated with final song accuracy (all father song: r(10) = - 0.752, p = 0.005; directed father song: r(10) = -0.648, p = 0.023) (Figure 4.4).

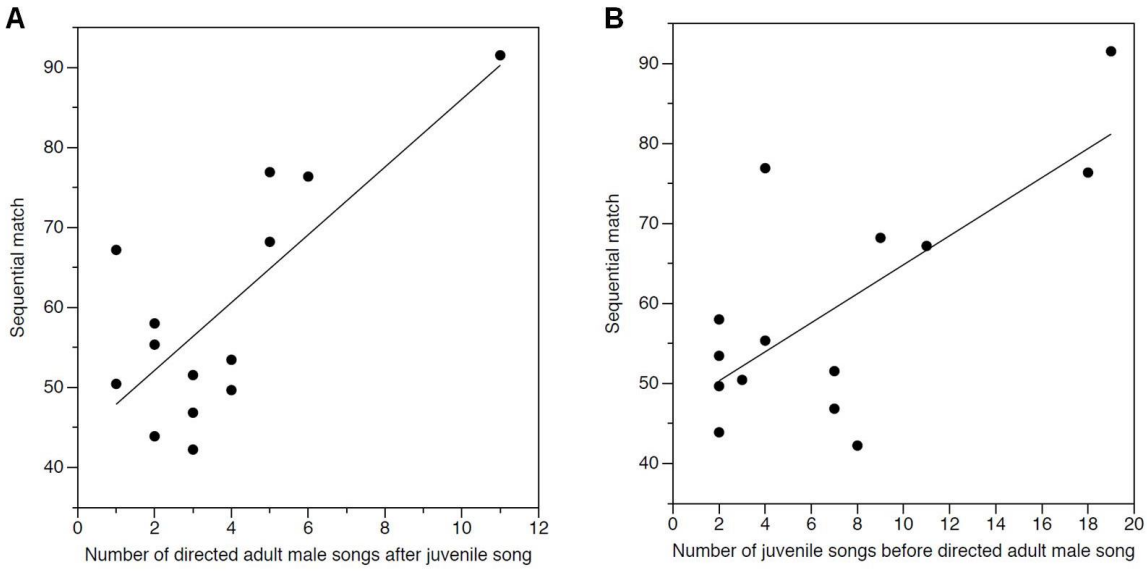

Figure 4.3. Directed paternal song after juvenile song predicts final song sequential match.
Whether analyzed based on number of instances of fathers singing contingently on juveniles, or the number of juvenile songs that preceded a paternal song, fathers singing after juveniles was positively correlated with final sequential match between fathers and juveniles. (A) The number of directed adult male songs *after* juvenile song bouts is significantly positively correlated with final sequential match between juvenile and tutor (r(10) = 0.734, p = 0.007). (B) The number of juvenile songs *before* directed adult male song is significantly positively correlated with final sequential match between juvenile and tutor (r(10) = 0.775, p = 0.003).

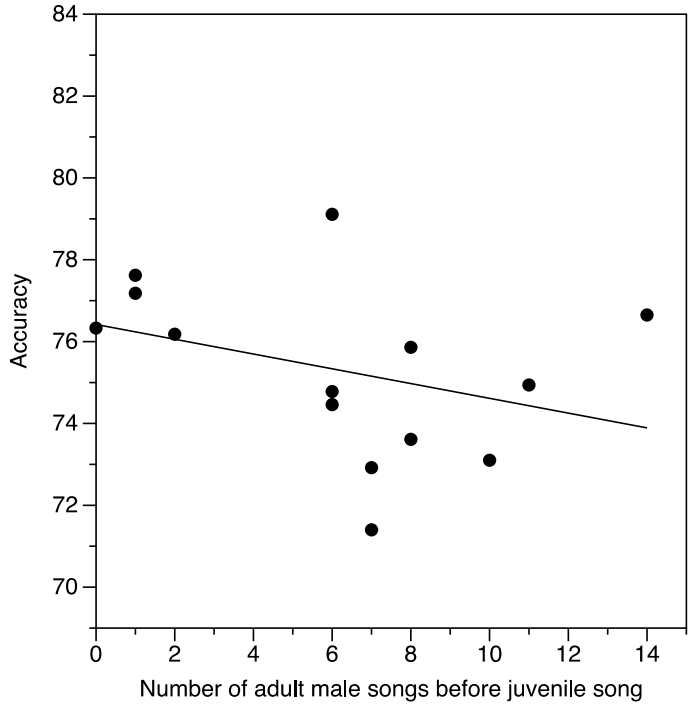

Figure 4.4. Directed and undirected paternal song before juvenile song is negatively correlated with final song accuracy.
There was a significant negative correlation between the total number of adult male songs (both directed and undirected) *before* juvenile song and accuracy of learning $r(10) = -0.752$, $p = 0.005$.

Female fluff-ups were associated with higher sequence similarity between juveniles and their fathers (total number of female fluff-ups around juvenile song: $r(10) = 0.866$, $p < 0.001$) (Figure 4.5). Fluff-ups predicted sequential similarity regardless of whether they occurred before ($r(10) = 0.938$, $p < 0.001$) or contingently on (during, after, or between: $r(10) = 0.837$, $p = 0.001$) juvenile male songs. Maternal fluff-ups after or between juvenile songs also predicted final percent similarity to tutor ($r(10) = 0.581$, $p = 0.047$). Contingent female wing strokes across the developmental period were not associated with any of the song outcome measures.

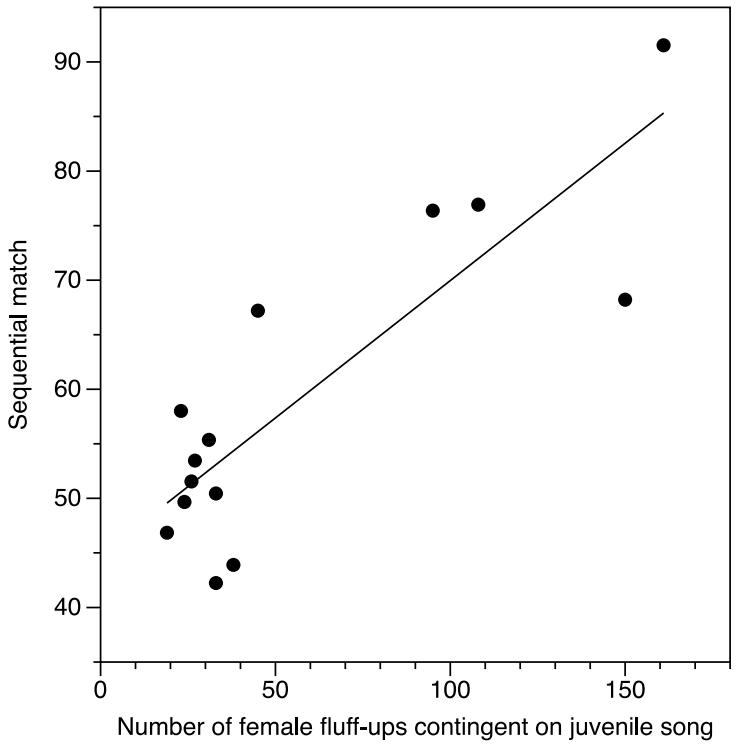

Figure 4.5. Contingent maternal fluff-ups predict final song sequential match.
The total number of female fluff-ups during, after, or between juvenile songs was significantly positively correlated with final sequential match between juvenile and tutor ($r(10) = 0.837$, $p = 0.001$).

No song outcome measure was significantly correlated with the total amount or rate of paternal song juveniles were exposed to across development, nor the amount or rate of song produced by the juveniles ($ps > 0.05$). Song outcomes were also not correlated with the number of male siblings ($p > 0.05$).

Relative contributions of family members to song learning

To examine the joint contributions of various behaviors to song learning outcomes, we performed a principal component analysis constructed from paternal song behavior, juvenile song timing, and maternal feedback behaviors (Table 2), using

Varimax rotation. This resulted in a PCA with five components with Eigenvalues above 1. The first component had an Eigenvalue of 8.086 and accounted for 24.79% of variance, and loaded most heavily on maternal fluff-up behaviors, paternal directed song, and juvenile singing around paternal directed song. A partial correlation revealed significant correlations between the first component and the learning outcome measures of percent similarity ($r(11) = 0.64$, $p = 0.019$) and sequential match ($r(11) = 0.854$, $p < 0.001$).

Table 4.2. Principal component analysis constructed from paternal song behavior, juvenile song timing, and maternal feedback behaviors.
Behaviors with the strongest contribution to each component are marked in bold. PCA Factor 1, loaded most heavily with maternal fluff-ups, directed adult song after juvenile song, and juvenile song before directed adult song, was significantly correlated with final song percent similarity to tutor ($r = .640$, $p = .019$), sequential match ($r = .854$, $p < .0001$) and overall similarity ($r = .902$, $p < .0001$), and was used to guide subsequent factor analyses. AS = adult song, JS = juvenile song, WS = wing stroke, FU = fluff-up.

	Components derived				
	1	***2***	***3***	***4***	***5***
Number of WS before JS	0.163	-0.045	**0.972**	0.024	-0.042
Number of WS during JS	0.179	-0.003	**0.952**	-0.040	-0.076
Number of WS between JS	0.064	0.099	**0.920**	0.021	-0.222
Number of WS after JS	0.161	0.069	**0.960**	-0.077	-0.024
Number of FU before JS	**0.865**	-0.122	0.245	0.213	-0.005
Number of FU during JS	**0.865**	0.075	0.470	-0.042	0.002

Number of FU between JS	**0.932**	0.084	0.298	0.044	-0.116
Number of FU after JS	**0.836**	-0.031	0.192	0.149	0.039
Number of Undirected AS before JS	-0.004	**0.790**	0.394	-0.058	0.069
Number of Undirected AS during JS	-0.147	**0.816**	0.017	-0.104	0.516
Number of AS between JS	0.364	**0.871**	-0.092	0.149	0.211
Number of Undirected AS after JS	0.451	0.386	**0.678**	-0.077	0.124
Number of Directed AS before JS	0.136	-0.033	0.010	**0.880**	0.153
Number of Directed AS during JS	0.466	0.019	-0.269	0.460	**0.641**
Number of Directed AS after JS	**0.885**	0.335	-0.099	0.077	0.119
Number of JS before undirected AS	0.398	**0.841**	0.021	0.237	-0.106
Number of JS during undirected AS	-0.148	0.362	-0.149	0.233	**0.832**
Number of JS after undirected AS	-0.004	**0.942**	0.165	0.050	-0.126

Number of JS between total AS	0.073	**0.935**	-0.050	0.058	0.253
Number of JS before directed AS	**0.677**	0.451	-0.037	0.300	-0.176
Number of JS during directed AS	-0.066	0.456	-0.323	0.561	0.245
Number of JS after directed AS	0.525	0.185	0.069	**0.788**	0.023
Eigenvalue	5.453	5.404	4.897	2.255	1.727
% of Variance	24.787	24.564	22.260	10.252	7.848

To determine which aspects of this component were most strongly correlated with which outcome measures, we then performed three additional factor analyses separately comparing adult song behavior to juvenile song behavior (Adult Song factor analysis, 'AS'), juvenile song rate and timing in relation to paternal song (Juvenile Song factor analysis, 'JS'), and maternal wing-strokes and fluff-ups to juvenile song (Maternal Gestures factor analysis, 'MG') (Table 3), and ran partial correlations between the resulting factors and learning outcomes. These analyses showed that the second principal component (PC2) of each analysis was associated with juvenile learning outcomes: directed adult song produced before or during juvenile song (PC2 of analysis AS), all maternal fluff-ups (PC2 of analysis MG), and juvenile song produced near in time, but not during, adult directed song (PC2 of analysis JS) each contribute to different aspects of song learning. PC2 of analysis MS (Eigenvalue 2.131; 30.44% of variance) was negatively correlated with final song

accuracy ($r(11) = -0.612$, $p = 0.026$) (Figure 4.6A), more firmly reiterating our finding that adult song produced before juvenile song is negatively correlated with learning outcomes. PC2 of analysis MG (Eigenvalue 3.475; 43.44% of variance) was correlated with sequential match ($r(11) = 0.863$, $p < 0.001$) (Figure 4.6B) and percent similarity ($r(11) = 0.583$, $p = 0.037$), reinforcing the finding that fluff-ups are a reinforcement signal of song at the level of syntax. PC2 of analysis JS (Eigenvalue 1.951; 27.88% of variance) was positively correlated with sequential match ($r(11) = 0.661$, $p = 0.014$) (Figure 4.6C and 4.6D), suggesting that the timing of juvenile's song in relation to their fathers' directed song affects how well they learn note order.

Table 4.3. Components derived from principal component analysis based on adult song relative to juvenile song, juvenile song relative to adult song, and female gestures relative to juvenile song.
Behaviors with the strongest contribution to each component are marked in bold. Based on the most predictive factors found in the initial PCA analysis (see Table 4.2), factors based on directed adult song and maternal fluff-ups were selected as explaining the greatest variance in the data. AS = adult song, JS = juvenile song, WS = wing stroke.

Factor analysis 'AS': Adult male song timing	Components derived 1AS	2AS
Number of Undirected AS before JS	**0.904**	-0.139
Number of Undirected AS during JS	**0.809**	0.033
Number of AS between JS	**0.804**	0.461
Number of Undirected AS after JS	**0.720**	0.106
Number of Directed AS during JS	0.057	**0.903**
Number of Directed AS	-0.100	**0.781**

before JS		
Number of Directed AS after JS	0.442	**0.679**
Eigenvalue	2.844	2.131
% of Variance with Rotation	40.62	30.44

Factor analysis 'JS': Juvenile song timing	Components derived 1JS	2JS	3JS
Number of JS after undirected AS	**0.964**	0.080	0.130
Number of JS between total AS	**0.893**	0.256	0.332
Number of JS before undirected AS	**0.836**	0.485	0.095
Number of JS after directed AS	0.116	**0.865**	0.236
Number of JS before directed AS	0.365	**0.836**	-0.054
Number of JS during undirected AS	0.172	-0.065	**0.925**
Number of JS during directed AS	0.185	0.440	**0.756**
Eigenvalue	2.636	1.951	1.622
% of Variance with Rotation	37.66	27.88	23.18

Factor analysis 'MG': WS and Fluff-ups	Components derived 1MG	2MG

relative to JS		
Number of WS between JS	**0.964**	0.120
Number of WS before JS	**0.959**	0.243
Number of WS during JS	**0.949**	0.255
Number of WS after JS	**0.934**	0.239
Number of Fluff –ups between JS	0.248	**0.942**
Number of Fluff –ups before JS	0.188	**0.898**
Number of Fluff –ups after JS	0.069	**0.896**
Number of Fluff –ups during JS	0.404	**0.884**
Eigenvalue	3.887	3.475
% of Variance with Rotation	48.59	43.44

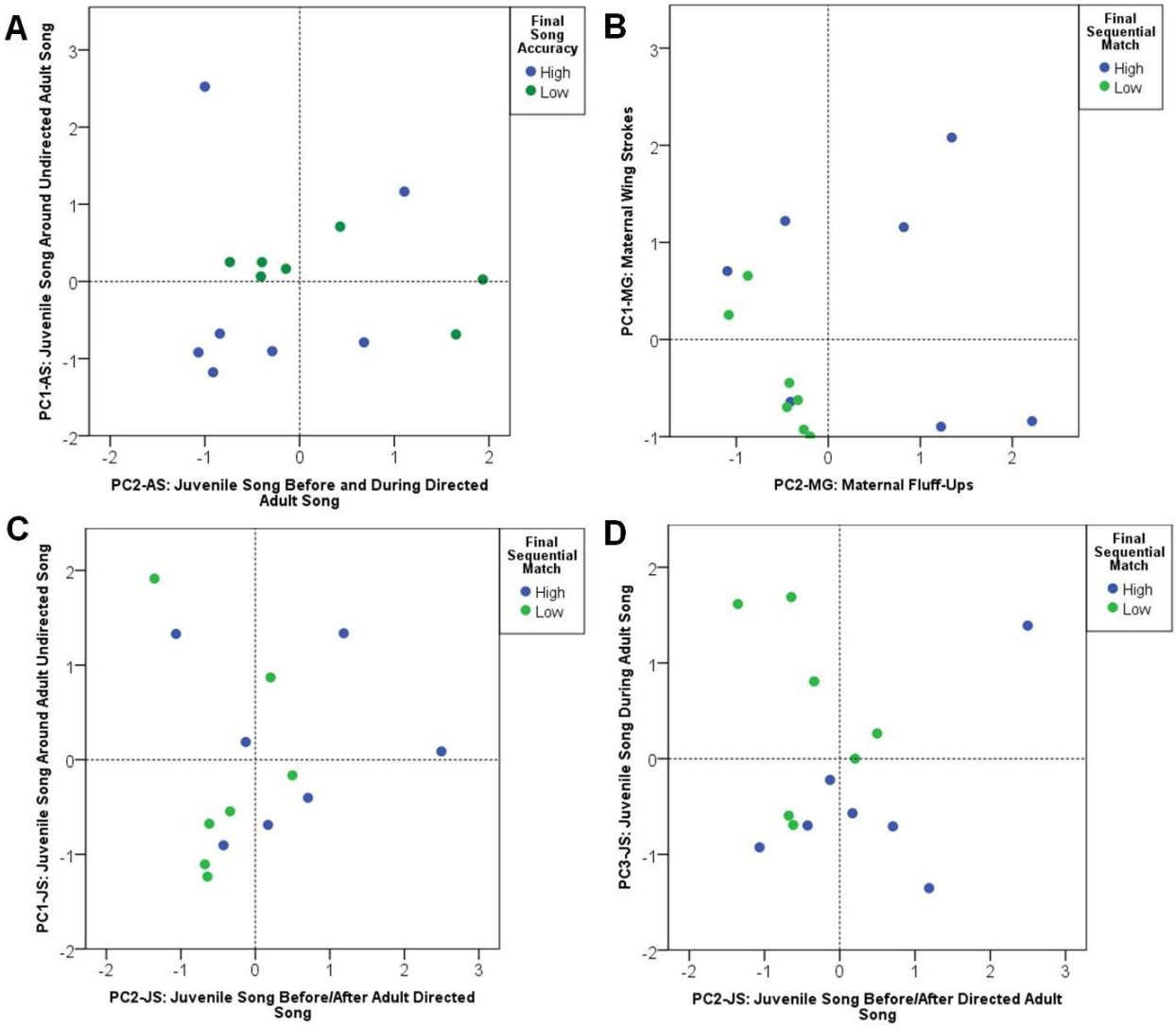

Figure 4.6. Principal component analyses demonstrate that timing and form of maternal and paternal behaviors jointly contribute to song learning.
'High' and 'Low' values for each song outcome measure were defined as falling above or below the median value, respectively. The second principal component (PC2) of each analysis was found to be associated with juvenile learning outcomes. For weightings of each component, see Table 4.3. (A) The adult song analysis (analysis AS) was loaded with the timing of directed and undirected adult song in relation to juvenile song. PC2-AS was heavily composed of directed song before and during juvenile song, and was significantly negatively correlated with final song accuracy ($r(11) = -0.612$, $p = 0.026$), unlike PC1-AS which was composed of juvenile song before, during, and after undirected adult song and was not correlated with any song learning measures. This suggests that directed paternal song after before and during juvenile song had a negative impact on learning outcomes. (B) The maternal behaviors analysis (analysis MG) was loaded with the timing of the female feedback behaviors of wing strokes and fluff-ups. PC2-MG was heavily composed of fluff-ups before, after, and during juvenile song, and was significantly correlated with final song sequential match ($r(11) = 0.863$, $p < 0.001$) and percent similarity ($r(11) = 0.583$, $p = 0.037$). This suggests that

contingent fluff-ups are a reinforcement signal of song at the level of syntax. PC1-MG, which was composed of wing strokes, was not correlated with any outcome measures. (C) The juvenile song analysis (analysis JS) was loaded with the timing of juvenile song in relation to adult directed and undirected song. PC2-JS was heavily composed of juvenile song before and after adult song, and was significantly correlated with final song sequential match ($r(11) = 0.661$, $p = 0.014$). PC1-JS, which was composed largely of juvenile song before, during, and after undirected adult song, was not correlated with any song outcomes. This suggests that juveniles which sang near in time to adult directed song, rather than undirected song, had superior learning outcomes. (D) PC3-JS, which was composed of juvenile song during adult directed and undirected song, was also not correlated with any outcome measures. This suggests that juveniles which 'interrupted' adult song with their own singing did not learn as well as those which sang before or after adult songs.

Discussion

We investigated the type and amount of social feedback occurring around subsong and plastic song of juvenile zebra finches by exploring the microstructure of social interactions. We found that contingent interactions with both parents were correlated with the song learning process and with fidelity of learning in the mature song. Maternal feedback influenced song learning, as juveniles experiencing more fluff-ups in temporal proximity to their own singing learned their father's song sequence more accurately. Given that female fluff-ups presented contingently on song in an experimental paradigm facilitate song learning (Carouso-Peck & Goldstein, 2019a), our finding suggests that juveniles also naturally use contingent maternal fluff-ups as cues which reinforce their song structure.

Paternal feedback also influenced song learning. Juveniles whose immature song received more contingent directed song from their fathers learned more similar copies of their father's song, and learned his sequence more correctly. Conversely, increased juvenile singing after the father's song predicted less accurate learning. Why do juveniles learn from contingent feedback provided by the father, rather than

by singing after the presentation of a song model? According to the action-based learning model, juvenile songbirds may adjust their repertoire depending on vocal interactions and retain elements that elicit reactions from the receivers, such as their territorial neighbors or their father (Nelson & Marler, 1994). It is also possible that contingent singing helps to organize juvenile attention, or that adults simplify their song in the presence of juveniles, making it easier to reproduce (Chen, Matheson & Sakata, 2016).

Directed song by the father was an especially powerful signal, as juvenile learning outcome was predicted by directed tutor song rather than undirected singing, despite being produced about one third as often in conjunction with immature song. Courtship song may be a more salient communication signal than undirected singing for a number of reasons: it is frequently accompanied by dynamic choreography aimed at the female (Williams, 2001) and is delivered with a faster tempo and less spectral variability than undirected song (Kao & Brainard, 2006; Sossinka & Böhner, 1980). Moreover, the song of adult males elicited more wing strokes from adult females than were elicited by juvenile song. As both contingent song from adult males and fluff-ups from adult females predict song learning, these behaviors may be working in conjunction to improve juvenile learning, as suggested by our principal component analyses which show maternal and paternal behaviors jointly contribute to song sequence learning.

Comparing the efficacy of the different forms of social feedback on vocal learning, a principal component analysis revealed that maternal fluff-ups, paternal female-directed song, and juvenile song produced near in time to paternal song, jointly

predict percent similarity and sequential match. Maternal fluff-ups were correlated with sequential match and percent similarity, while paternal song produced after juvenile song, but not before, predicted sequential match. Family identity accounted for a large proportion of variance in the data, and it is possible that differences in the behavior of the mothers and fathers explain the observed effects.

Our results emphasize the importance of microanalytic approaches to quantifying the form and timing of behavior. Analyzing the moment-to-moment dynamics of social interaction reveals infrequent behaviors around juvenile song that may have a strong impact on the learning process. Such an approach has proven useful for investigating the dynamic structure of multimodal parent-infant interactions on early speech and language learning (e.g. Goldstein, King, & West, 2003; Smith, Yu, & Pereira, 2011; Suarez-Rivera, Smith & Yu, 2019). In our data set, most of the contingent paternal and maternal behaviors occurred infrequently. Only 1.3% of juvenile songs elicited a maternal fluff-up. Visual feedback cues to juveniles from female cowbirds are also extremely rare behaviors, as on average 1.1 wing strokes occur to every 100 juvenile songs (West & King, 1988). About 4 % of juvenile song elicited feedback from adult males, and directed song by the father produced contingently on juvenile song accounted for less than 2% of total adult male song. Nevertheless, these types and rates of social cues robustly shape juvenile cowbird song, and change its content as well as its developmental trajectory (Smith, King, & West, 2000), similar to the effect we observed with zebra finch maternal fluff-ups.

How could such infrequent parental responses become so salient for learning? Given the presence of an adult male, song is a ubiquitous feature of a young bird's

environment, creating numerous opportunities for learning. For example, juveniles may learn from observing social interactions among other adults (Pepperberg, 1985). Female brown-headed cowbirds eavesdrop on interactions between other males and females and use the information available in the social group for preference formation (Gros-Louis et al., 2003). Juvenile zebra finches appear to be learning some acoustic features simply from exposure to tutor song (e.g. Eales, 1985; Rodríguez-Santos, 2017; Derégnaucourt, 2013), but accurate learning requires some form of contingent feedback, whether from tutors (Chen, Matheson & Sakata, 2016), from females (Adret, 2003; Carouso-Peck & Goldstein, 2019a) or from actions such as pecking a key that triggers song (Tchernichovski et al., 2001; Rodríguez-Saltos, 2017).

Parental responsiveness may also vary over developmental time, such that there may be periods of more frequent feedback. We have assessed the overall impact of parental feedback on the final song outcome of the juvenile males, but have not analyzed how parental feedback may differentially affect song learning when received at specific times in development. While we did not find wing strokes to predict song outcomes when pooled across development, it is possible that they are relevant only during a specific window. To uncover such developmental specificity, we are currently observing changes in juvenile singing activity and in the associated parental feedback as they unfold over time.

Taken together, exposure to multisensory information (song of the adult male, visual signals from the adult female) may organize juvenile perception and enhance learning via increased attention to social cues (Chen, Matheson & Sakata, 2016) or arousal (Carouso-Peck & Goldstein, 2019a; Carouso-Peck & Goldstein, 2018), effects

that may be enhanced if the signals co-occur. Exposure to concurrent auditory and visual stimuli has been shown to enhance learning in nightingales, as juveniles exposed to stroboscope flashes during tutoring developed both larger repertoires and produced better copies of the song model than controls exposed only to the tutor songs (Hultsch, Schleuss, & Todt, 1999). Human infants appear to use a similar mechanism. Infants aged 7.5 months use visual information that is synchronized with the speech stream to aid perceptual segmentation (Hollich, Newman, & Jusczyk, 2005; Gogate & Bahrick, 1998).

Our results augment the dominant model of song learning with socially-embedded mechanisms for feedback and learning. The dominant model describes song development in terms of an early sensory memorization phase followed by a later sensorimotor practice phase that is based on recalled memories of the tutor song (Konishi, 1965; Marler, 1976). Such a model of song learning may be appropriate for territorial songbirds, in which the two phases are typically temporally distinct. Immature vocalizations develop through a process of being compared to the mature songs of territorial neighbors. Passive "eavesdropping" on vocal duels between territorial neighbors facilitates song learning (Beecher et al., 2007). In contrast, the sensory and sensorimotor phases overlap in time for zebra finches and brown-headed cowbirds, creating the developmental opportunity for social feedback on their immature song to update an incomplete song representation. These species are highly gregarious and non-territorial, allowing for social interactions during development which may influence learning. Given the diversity of life history strategies and developmental trajectories of song learning across passerines, a single model of song

learning may not generalize across species (Beecher & Brenowitz, 2005; DeVoogd, 2004).

Consequently, the biology of a particular species and its specific social environment must always be considered when studying mechanisms of song acquisition. In the zebra finch, juvenile vocalizations elicit the feedback of conspecifics, and come in response to actions of conspecifics. Developing individuals therefore play an active role in a communication and learning feedback loop. As the zebra finch is socially gregarious, close proximity to conspecifics allows for rapid interchanges involving multiple sensory modalities, which may explain why passive tape tutoring results in impoverished learning compared to live interactions (Derégnaucourt et al., 2013). Moreover, zebra finches are motivated to interact socially, as conspecifics have positive valence in contrast to related territorial finch species (Goodson et al., 2005; Goodson & Thompson, 2010). The song learning mechanisms at work in the zebra finch may be deeply embedded in a brain shaped by social functions, drawing on circuitry that is also used for social motivation and reward (e.g. Baran & Peck et al., 2017; Carouso-Peck & Goldstein, 2018).

Wing strokes are believed to be precursors of the copulation-solicitation preference display in cowbirds. Juveniles seem to be able to take advantage of female signals that evolved for a different function, a process called “inadvertent coaching” (Hoppitt & Laland, 2008). The developmental process bears striking similarity to vocal learning in human infants. Infant babbling elicits rapid contingent social responses from caregivers, and these responses influence infant vocal learning in real time and developmental time (Goldstein, Schwade, & Bornstein, 2009; Albert,

Schwade & Goldstein, 2017; Goldstein & Schwade, 2008; Elmlinger, Schwade & Goldstein, in press). In a strong parallel with the songbird findings, contingent parental feedback can induce more developmentally-advanced vocalizations even when it consists only of non-vocal responses such as touching, moving closer or smiling at the infants (Goldstein, King & West, 2003).

Our results support the existence of a dynamic system of socially guided learning, in which senders and receivers alternate roles as they participate in multisensory information exchange. We have shown that paternal song produced after, but not before, juvenile song predicts positive learning outcomes, contrary to what would be predicted by a traditional imitation model. We have previously shown that female fluff-ups presented contingently on juvenile song production result in more accurate song learning in a video playback paradigm (Carouso-Peck & Goldstein, 2019a) and have now demonstrated a similar effect in a naturalistic context in which mothers selectively responded to their sons. In summary, both parents contribute to successful song development. In nature, juvenile songbirds can rely on parental feedback, because their altricial state requires the presence of caregivers. Thus young songbirds are born into a structured social environment, with the essential developmental task of extracting information from it.

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REFERENCES

Adret, P. (2003). Vocal imitation in blindfolded zebra finches (*Taeniopygia guttata*) is facilitated in the presence of non-singing females. *Journal of Ethology, 22*, 29-35.

Akçay, C., Tom, M.E., Campbell, S.E., & Beecher, M.D. (2013). Song type matching is an honest threat signal in a hierarchical animal communication system. *Proceedings of the Royal Society B, 280,* 201222517.

Albert, R., Schwade, J. A., & Goldstein, M. H. (2017). The social functions of babbling: Acoustic and contextual characteristics that facilitate maternal responsiveness. Developmental Science, 18, 1-11.e12641

Anderson, R. C. (2009). Operant conditioning and copulation solicitation display assays reveal a stable preference for local song by female swamp sparrows Melospiza georgiana. *Behavioral Ecology and Sociobiology, 64*(2), 215-223.

Baran, N. M., Peck, S. C., Kim, T. H., Goldstein, M. H., & Adkins-Regan, E. (2017). Early life manipulations of vasopressin-family peptides alter vocal learning. *Proceedings of the National Society B, 284*, 20171114.

Beecher, M. D., & Brenowitz, E. A. (2005). Functional aspects of song learning in songbirds. *Trends in Ecology & Evolution, 20*(3), 143-149.

Beecher, M. D., Burt, J. M., O'Loghlen, A. L., Templeton, C. N., & Campbell, S. E. (2007). Bird song learning in an eavesdropping context. *Animal Behaviour, 73*, 929-935.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological, 57*(1), 289-300.

Bohner, J. (1983). Song learning in the Zebra finch (*Taeniopygia-guttata*) - selectivity in the choice of a tutor and accuracy of song copies. *Animal Behaviour, 31*(1), 231-237.

Bolhuis, J. J., Okanoya, K., & Scharff, C. (2010). Twitter evolution: converging mechanisms in birdsong and human speech. *Nature Reviews Neuroscience, 11*(11), 747-759.

Carouso-Peck, S. & Goldstein, M. H (2018). Linking vocal learning to social reward in the brain: Proposed mechanisms of socially guided song learning. In *Oxford Handbook of Voice Perception*, Sascha Ühholz and Pascal Belin, Eds. Oxford University Press.

Carouso-Peck, S. & Goldstein, M. H. (2019a). Female social feedback reveals non-imitative mechanisms of vocal learning in the zebra finch. *Current Biology, 29*, 631-636.

Carouso-Peck, S. & Goldstein, M. H. (2019b). "A Bird's-Eye View of Communication". *Scientific American*, March 29.

Chen, Y., Matheson, L.E., & Sakata, J.T. (2016). Mechanisms underlying the social enhancement of vocal learning in songbirds. *Proceedings of the National Academy of Science, 113*, 6641-6646.

Clayton, N. S. (1987). Song tutor choice in Zebra finches. *Animal Behaviour, 35*(3), 714-721.

Derégnaucourt, S., Poirier, C., Kant, A.V., Linden, A.V., & Gahr, M. (2013). Comparisons of different methods to train a young zebra finch (*Taeniopygia guttata*) to learn a song. *Journal of Physiology – Paris, 107*, 210-218.

Deshpande, M., Pirlepesov, F. & Lints, T. (2014). Rapid encoding of an internal model for imitative learning. *Proceedings of the Royal Society B, 281*, 20132630.

DeVoogd, T. J. (2004). Where is the bird? *Annals of the New York Academy of Sciences, 1016*(1), 778-786.

Doupe, A. J., & Kuhl, P. K. (1999). Birdsong and human speech: common themes and mechanisms. *Annual Review of Neuroscience, 22*(1), 567-631.

Eales, L.A. (1985). Song learning in zebra finches: some effects of song model availability on what is learnt and when. *Animal Behaviour, 33*, 1293-1300.

Eales, L. A. (1987). Song learning in female-raised zebra finches: another look at the sensitive phase. *Animal Behaviour, 35*(5), 1356-1365.

Eales, L. A. (1989). The influences of visual and vocal interaction on song learning in zebra finches. *Animal Behaviour, 37*, 507-508.

Elmlinger, S. L., Schwade, J. A., & Goldstein, M. H. (in press). The ecology of prelinguistic vocal learning: Parents simplify the structure of their speech in response to babbling. *Journal of Child Language*.

Gogate, L. J. & Bahrick, L. E. (1998). Intersensory redundancy facilitates learning of arbitrary relations between vowel sounds and objects in seven-month-old infants. *Journal of Experimental Child Psychology, 69(2),* 133-149.

Goldstein, M. H., & Schwade, J. A. (2008). Social feedback to infants' babbling facilitates rapid phonological learning. *Psychological Science, 19*(5), 515-523.

Goldstein, M. H., King, A. P., & West, M. J. (2003). Social interaction shapes babbling: Testing parallels between birdsong and speech. *Proceedings of the National Academy of Sciences, 100*(13), 8030-8035.

Goldstein, M. H., Schwade, J. A., & Bornstein, M. H. (2009). The value of vocalizing: Five-month-old infants associate their own noncry vocalizations with responses from adults. Child Development, 80 (3), 636 – 644.

Goodson, J. L., & Thompson, R. R. (2010). Nonapeptide mechanisms of social cognition, behavior and species-specific social systems. *Current Opinion in Neurobiology, 20*(6), 784-794.

Goodson, J. L., Evans, A. K., Lindberg, L., & Allen, C. D. (2005). Neuro-evolutionary patterning of sociality. *Proceedings of the Royal Society B-Biological Sciences, 272*(1560).

Gros-Louis, J., White, D. J., King, A. P., & West, M. J. (2003). Female brown-headed cowbirds' (*Molothrus ater*) social assortment changes in response to male song: a potential source of public information. *Behavioral Ecology and Sociobiology, 53*(3), 163-173.

Healy, K., McNally, L., Ruxton, G. D., Cooper, N., & Jackson, A. L. (2013). Metabolic rate and body size are linked with perception of temporal information. *Animal Behaviour, 86*, 685-696.

Helekar, S. A., Marsh, S., Viswanath, N. S., & Rosenfield, D. B. (2000). Acoustic pattern variations in the female-directed birdsongs of a colony of laboratory-bred zebra finches. *Behavioural Processes, 49*(2), 99-110.

Hollich, G., Newman, R. S., & Jusczyk, P. W. (2005). Infants' use of synchronized visual information to separate streams of speech. *Child Development, 76*(3),

598-613.

Hoppitt, W., & Laland, K. N. (2008). Social processes influencing learning in animals: A review of the evidence. In H. J. Brockmann, T. J. Roper, M. Naguib, K. E. WynneEdwards, C. Barnard & J. C. Mitani (Eds.), *Advances in the Study of Behavior,* (Vol. 38, pp. 105-165).

Houx, B. B., & ten Cate, C. (1998). Do contingencies with tutor behaviour influence song learning in zebra finches? *Behaviour, 135*(5), 599-614.

Hultsch, H., & Todt, D. (1992). The serial order effect in the song acquisition of birds: relevance of exposure frequency to song models. *Animal Behaviour, 44*, 590-592.

Hultsch, H., Schleuss, F., & Todt, D. (1999). Auditory–visual stimulus pairing enhances perceptual learning in a songbird. *Animal Behaviour, 58*(1), 143-149.

Immelmann, K. (1969). Song development in the zebra finch and other estrildid finches. In R. A. Hinde, (Ed.), *Bird Vocalizations* (pp. 61–74), Cambridge Univ. Press, London.

Jones, A., & Slater, P. (1996). The role of aggression in song tutor choice in the zebra finch: Cause or effect? *Behaviour, 133*, 103-115.

Kao, M. H., & Brainard, M. S. (2006). Lesions of an avian basal ganglia circuit prevent context-dependent changes to song variability. *Journal of Neurophysiology, 96*(3), 1441-1455.

King, A. P., & West, M. J. (1977). Species identification in North-American cowbird - appropriate responses to abnormal song. *Science, 195*(4282), 1002-1004.

Konishi, M. (1965). The Role of Auditory Feedback in the Control of Vocalization in the White-Crowned Sparrow. *Zeitschrift fur Tierpsychologie, 22*(7), 770-783.

Mann, N. I., & Slater, P. J. B. (1994). What causes young male zebra finches, *Taeniopygia guttata*, to choose their father as song tutor? *Animal Behaviour, 47*(3), 671-677.

Mann, N. I., & Slater, P. J. B. (1995). Song tutor choice by zebra finches in aviaries. *Animal Behaviour, 49*(3), 811-820.

Mann, N. I., Slater, P. J. B., Eales, L. A., & Richards, C. (1991). The influence of

visual-stimuli on song tutor choice in the Zebra finch, Taeniopygia-guttata. *Animal Behaviour, 42*, 285-293.

Marler, P. (1976). Sensory templates in species specific behavior. In J. C. *Fentress, (Ed.), Simpler Networks and Behavior.* Illus. Sinauer Associates, Inc.: Sunderland, Mass., USA.

Marler, P., & Peters, S. (1982). Developmental overproduction and selective attrition: new processes in the epigenesis of birdsong. *Developmental Psychobiology, 15*, 369-78.

Menyhart, O., Kolodny, O., Goldstein, M. H., DeVoogd, T. J., & Edelman, S. (2015). Juvenile zebra finches learn the underlying structural regularities of their fathers' song. *Frontiers in Psychology, 6*, 571.

Nelson, D. A. & Marler, P. (1994). Selection-based learning in bird song development. *Proceedings of the National Academy of Sciences, 91*, 10498-10501.

Nowicki, S., Searcy, W. A., & Hughes, M. (1998). The territory defense function of song in song sparrows: A test with the speaker occupation design. *Behaviour, 135,* 615-628.

O'Loghlen, A. L., & Beecher, M. D. (1997). Sexual preferences for mate song types in female song sparrows. *Animal Behaviour, 53*(4), 835-841.

Pepperberg, I. M. (1985). Social modeling theory: A possible framework for understanding avian vocal learning. *The Auk, 102*(4), 854-864.

Price, P. H. (1979). Developmental determinants of structure in zebra finch song. *Journal of Comparative and Physiological Psychology, 93*(2), 260-277.

Roberts, T. F., Gobes, S. M. H., Murugan, M., Olveczky, B. P., & Mooney, R. (2012). Motor circuits are required to encode a sensory model for imitative learning. *Nature Neuroscience, 15,* 1454-1459.

Rodríguez-Saltos, C.A. (2017) To become senders, songbirds must be receivers first. *Integrative and Comparative Biology*, *57*(4), 910-919.

Roper, A., & Zann, R. (2006). The onset of song learning and song tutor selection in fledgling zebra finches. *Ethology, 112*(5), 458-470.

Scharff, C., & Nottebohm, F. (1991). A comparative-study of the behavioral deficits

following lesions of various parts of the zebra finch song system - implications for vocal learning. *Journal of Neuroscience, 11*(9), 2896-2913.

Searcy, W. A., & Marler, P. (1981). A test for responsiveness to song structure and programming in female sparrows. *Science, 213*(4510), 926-928.

Slater, P. J. B., Eales, L. A., & Clayton, N. S. (1988). Song learning in zebra finches (Taeniopygia guttata): progress and prospects. *Advances in the Study of Behavior, 18*,1-34.

Smith, L. B., Yu, C., & Pereira, A. F. (2011). Not your mother's view: the dynamics of toddler visual experience. *Developmental Science, 14*(1), 9-17.

Smith, V. A., King, A. P., & West, M. J. (2000). A role of her own: female cowbirds, Molothrus ater, influence the development and outcome of song learning. *Animal Behaviour, 60*, 599-609.

Soha, J. A., & Marler, P. (2001). Vocal syntax development in the white-crowned sparrow (Zonotrichia leucophrys). *Journal of Comparative Psychology, 115(2)*, 172-180.

Sossinka, R., & Böhner, J. (1980). Song Types in the Zebra Finch Poephila guttata castanotis. *Zeitschrift fur Tierpsychologie, 53*(2), 123-132.

Sturdy, C. B., Phillmore, L. S., & Weisman, R. G. (1999). Note types, harmonic structure, and note order in the songs of zebra finches (Taeniopygia guttata). *Journal of Comparative Psychology, 113*(2), 194-203.

Suarez-Rivera, C., Smith, L. B., & Yu, C. (2019). Multimodal parent behaviors with joint attention support sustained attention in infants. *Developmental Psychology, 55*(1), 96-109.

Tchernichovski, O., & Nottebohm, F. (1998). Social inhibition of song imitation among sibling male zebra finches. *Proceedings of the National Academy of Sciences, 95*(15), 8951-8956.

Tchernichovski, O., Mitra, P. P., Lints, T., & Nottebohm, F. (2001). Dynamics of the vocal imitation process: How a zebra finch learns its song. *Science, 291*, 2564-2569.

Tchernichovski, O., Nottebohm, F., Ho, C. E., Pesaran, B., & Mitra, P. P. (2000). A procedure for an automated measurement of song similarity. *Animal*

Behaviour, 59, 1167-1176.

Tchernichovski, O., Swigger, D., & Partha, M. (2004). Sound Analysis Pro User Manual. CCNY, New York.

ten Cate, C. (1991). Behaviour-contingent exposure to taped song and zebra finch song learning. *Animal Behaviour, 42*(5), 857-859.

Vallet, E., & Kreutzer, M. (1995). Female canaries are sexually responsive to special song phrases. *Animal Behaviour, 49*(6), 1603-1610.

Vyas, A., Harding, C., Borg, L., & Bogdan, D. (2009). Acoustic characteristics, early experience, and endocrine status interact to modulate female zebra finches' behavioral responses to songs. *Hormones and Behavior, 55*(1), 50-59.

West, M. J. & King, A. P. (1985). Social guidance of vocal learning by female cowbirds: Validating its functional significance. *Zeitschrift fur Tierpsychologie, 70,* 225-235.

West, M. J., & King, A. P. (1988). Female visual-displays affect the development of male song in the cowbird. *Nature, 334*(6179), 244-246.

Williams, H. (1990). Models for song learning in the zebra finch: fathers or others? *Animal Behaviour, 39*(4), 745-757.

Williams, H. (2001). Choreography of song, dance and beak movements in the zebra finch (Taeniopygia guttata). *Journal of Experimental Biology, 204*(20), 3497-3506.

Williams, H. (2004). Birdsong and singing behavior. *Annals of the New York Academy of Sciences, 1016,* 1-30.

Wittenburg, P., Brugman, H., Russel, A., Klassmann, A., and Sloetjes, H. (2006). ELAN: a professional framework for multimodality research. In Proceedings of LREC, Fifth International Conference on Language Resources and Evaluation.

Zann, R. A. (1990). Song and call learning in wild zebra finches in south-east Australia. *Animal Behaviour, 40*(5), 811-828.

Zann, R. A. (1996). The zebra finch: A synthesis of field and laboratory studies. Oxford University Press. New York.

CHAPTER 5

EARLY LIFE MANIPULATIONS OF VASOPRESSIN-FAMLY PEPTIDES ALTER VOCAL LEARNING

Abstract

Vocal learning from social partners is crucial for the successful development of communication in a wide range of species. Social interactions organize attention and enhance motivation to learn species-typical behavior. However, the neurobiological mechanisms connecting social motivation and vocal learning are unknown. Using zebra finches (*T. guttata*), a ubiquitous model for vocal learning, we show that manipulations of nonapeptide hormones in the vasopressin family [arginine vasotocin (AVT)] early in development can promote or disrupt both song and social motivation. Young male zebra finches, like human infants, are socially gregarious and require interactive feedback from adult tutors to learn mature vocal forms. To investigate the role of social motivational mechanisms in song learning, in two studies we injected hatchling males with AVT or Manning Compound (MC, a nonapeptide receptor antagonist) on days 2-8 post-hatching and recorded song at maturity. In both studies, MC males produced a worse match to tutor song than controls. In Study 2, the AVT males learned song significantly better compared to controls. Furthermore, song similarity was correlated with several measures of social motivation throughout development. These findings provide the first evidence that nonapeptides are critical to the development of vocal learning.

Introduction

From the earliest stages, language development in humans is guided by social interaction. For example, infants' prelinguistic vocalizations facilitate parental responses (Goldstein & West, 1999), and infants use those reactions to refine their vocal repertoires to match those of the ambient language (Goldstein, King & West, 2003; Goldstein & Schwade, 2008). Attention to social responses is thus an important component of vocal learning, and developmental disorders that affect social motivation, such as autism spectrum disorder (ASD), are associated with deficits in prelinguistic vocal development (Warlaumont et al., 2014; Chevallier et al., 2012). What mechanisms link social motivation and vocal learning? The neuroendocrine processes underlying affiliative behavior may also mediate social influences on communicative development, but previous studies of vocal learning have not incorporated candidate neuroendocrine mechanisms. Social influences on vocal development are present in other vocal learners, such as songbirds (West & King, 1988; Kojima, Doupe & Knudsen, 2011; Ljubičić, Hyland Bruno & Tchernichovski, 2016; Chen, Matheson & Sakata, 2016), but specific pathways linking social interaction to developmental changes in song are not known.

Song learning in birds has become a ubiquitous model for understanding general principles underlying complex vocal learning across species, including language learning in humans (Goldstein & Schwade, 2009; Lipkind et al., 2013). Zebra finches (*Taeniopygia guttata*), like human infants, require interactive feedback from adult tutors to learn mature vocal forms (Derégnaucourt et al., 2013; Slater, Eales & Clayton, 1988). Zebra finches are highly gregarious and experience a high degree of

temporal overlap in the memorization and acquisition phases of song learning (Roper & Zann, 2006), allowing social processes to influence learning. Social interaction with a tutor is vital for normal song development (Price, 1979; Williams, Kilander & Sotanski, 1993; Williams, 2004) and young zebra finches cannot learn effectively from a passive tape-recorded song (Derégnaucourt et al., 2013; Eales, 1989). Zebra finches cross-fostered under Bengalese finches (*Lonchura striata*) will produce a good copy of their foster-parent's song, even if a zebra finch model is available in a neighboring cage (Immelmann, 1969; Böhner, 1983). Non-singing female listeners are also known to affect song learning in the zebra finch (Jones & Slater, 1993). Males raised with deaf adult females sing more frequently and develop more atypical songs than those raised with hearing females (Williams, 2004) and blindfolded males raised with a tutor develop more accurate song when also raised with a female sibling than without one (Adret, 2003). While both vocal learning and neuroendocrine mechanisms of social behavior have been investigated in the zebra finch, they have never been integrated.

It is well established that nonapeptide hormones in the vasopressin family [arginine vasopressin (AVP) and oxytocin (OT) in mammals; arginine vasotocin (AVT) and mesotocin (MT) in birds, reptiles, and amphibians] are involved in social, motivational, sensory, and motor processes, all of which may support vocal learning from social partners. These small peptide hormones, which derive from hypothalamic and smaller accessory cell groups, modulate social behaviors across taxa and have been identified as mediators of behavioral plasticity and diversity (Goodson, 2005; O'Connell & Hofmann, 2011; Insel, 2010; Insel &Young, 2001). Changes to vocal

behavior are among the most common and pronounced effects of nonapeptides. AVT/AVP affects latency, duration, and acoustic features of vocalizations in several vertebrate species, including fish (Goodson & Bass, 2000a; Goodson & Bass, 2000b), amphibians (Boyd, 2013), rodents (Lukas & Wöhr, 2015; Scattoni et al., 2008), and birds (Voorhuis, De Kloet & De Wied, 1991; Harding & Rowe, 2003; Maney, Goode & Wingfield, 1997; Goodson, 1998; Goodson, Rinaldi & Kelly, 2009). However, the effect of nonapeptides on vocal learning in social contexts is unknown.

In two separate experiments, we manipulated the nonapeptide system of zebra finch chicks on 2-8 days post-hatch (dph) via daily intracranial (IC) injections of either AVT, Manning Compound (MC, a potent antagonist of the AVT/AVP 1a receptor [V1aR] and weak OT receptor antagonist) or a vehicle control and assessed the effect on song learning, specifically the acoustic match to the social father. The first experiment was designed to focus on the effect of nonapeptide treatment on social development and pairing behavior. Given the wide-ranging effect of treatment in the first study on a number of social behaviors, as well as adult song, we then designed the second experiment to more specifically focus on vocal learning in a naturalistic social environment. We predicted that AVT injected birds would show a better acoustic match to their social father's (tutor) song in adulthood than Controls, whereas MC males would show a worse match. We further predicted that MC males would exhibit social behavior deficits throughout development which would predict corresponding vocal learning deficits.

Results

a) Song Learning is Sensitive to Organizational Effects of Nonapeptides.

(i) Study 1

As predicted, nonapeptide treatment led to significant changes to males' crystalized song (Figure 5.1). Treatment affected the similarity score comparing subject and tutor song ($X^2(2) = 10.9$, $p = 0.004$) (Figure 5.2A). MC males had lower similarity than both Control and AVT males, but the difference between AVT and Control males did not reach significance. We found similar results for accuracy, a fine-grained measure of local similarity ($X^2(2) = 14.3$, $p = 0.0008$) (Figure 5.2B). There was no effect of treatment on the measure of sequential match ($X^2(2) = 0.8$, $p = 0.7$).

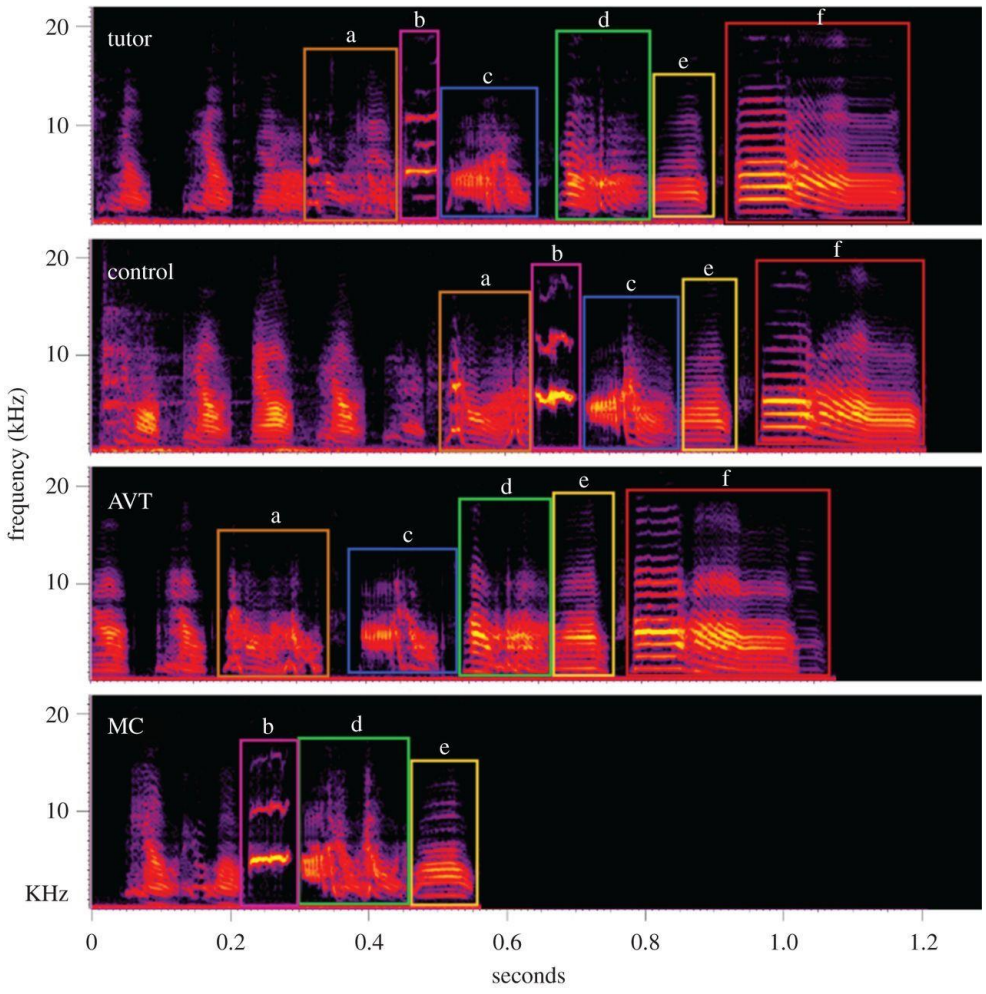

Figure 5.1. Example spectrograms of the song of a tutor male and three subject males from each treatment group.
Boxes outline individual song syllables. Letters label syllables that correspond between tutor and subject male song, as used for the syllable-level analyses in Study 2.

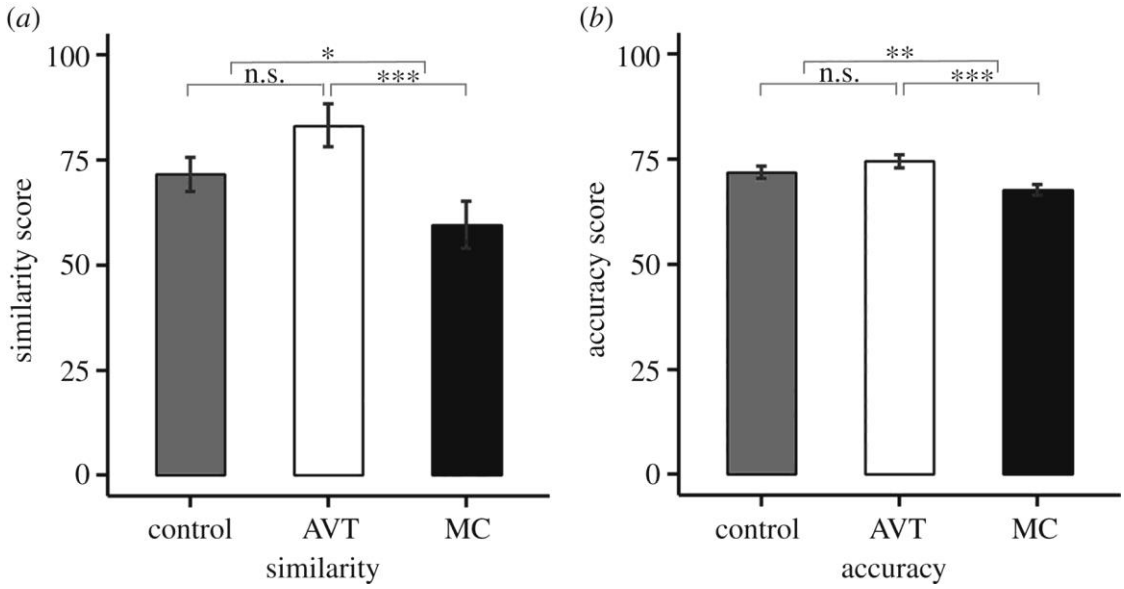

Figure 5.2. Study 1 similarity and accuracy scores at 90 dph.
(A) Mean ± SE similarity score for Study 1 males at day 90 when compared to social father's song as calculated using Sound Analysis Pro (SAP) (AVT-Control: p = 0.07; MC-Control: p = 0.03; AVT-MC: p = 0.0001) (B) Mean ± SE accuracy score for Study 1 males at day 90 (AVT-Control: p = 0.09; MC-Control: p = 0.001; AVT-MC: p < 0.0001). *p < 0.05, **p < 0.01, ***p < 0.001.

(ii) Study 2

The effects of nonapeptide treatment on song similarity from Study 1 were replicated and strengthened in the second study, which was designed to assess song development. Treatment predicted similarity both at 90 dph ($X^2(2) = 12.72$, $p = 0.002$; Figure 5.3A) and at 120 dph, when zebra finch song is fully crystalized ($X^2(2) = 11.16$, $p = 0.004$; Figure 5.3B). At both time points, all three treatment groups differed from each other, with AVT males having the highest similarity, MC males the lowest, and Control males intermediate. See Figure 5.4 for individual song similarity scores from both studies. Treatment did not impact either accuracy (90 dph: $X^2(2) = 1.6$, $p = 0.4$; 120 dph: $X^2(2) = 1.8$, $p = 0.4$) or sequential match (90 dph: $X^2(2) = 0.7$, $p = 0.7$); 120 dph: $X^2(2) = 1.3$, $p = 0.5$).

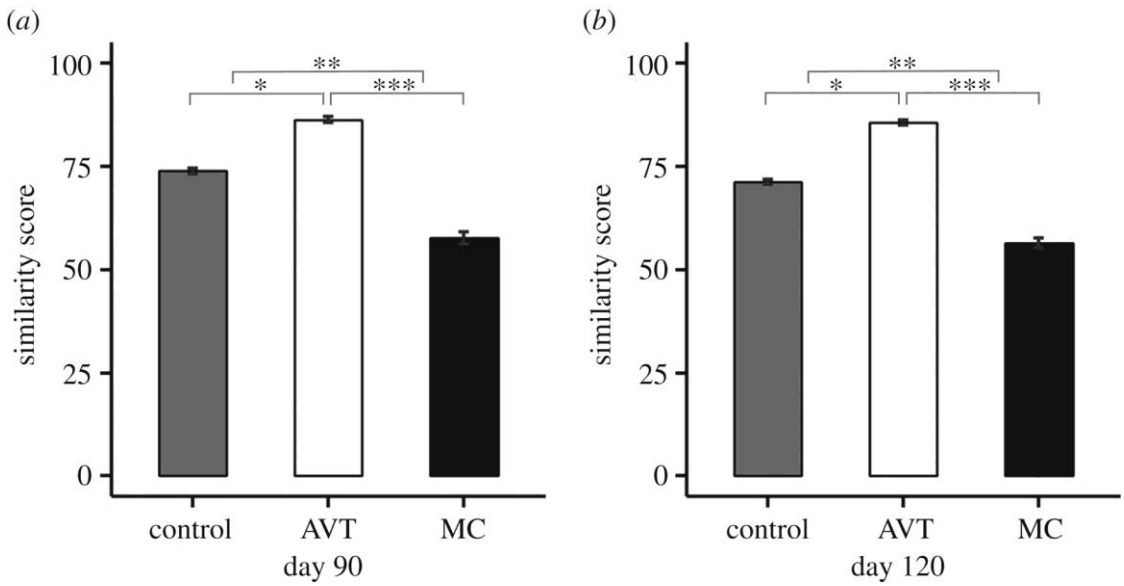

Figure 5.3. Study 2 similarity scores at 90 and 120 dph.
(A) Mean ± SE of the similarity score for Study 2 males at day 90 (AVT-Control: $p = 0.02$, MC-Control: $p = 0.006$; AVT-MC: $p < 0.0001$) (B) Mean ± SE of the similarity score for Study 2 males at day 120 (AVT-Control: $p = 0.02$, MC-Control: $p = 0.03$; AVT-MC: $p < 0.0001$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

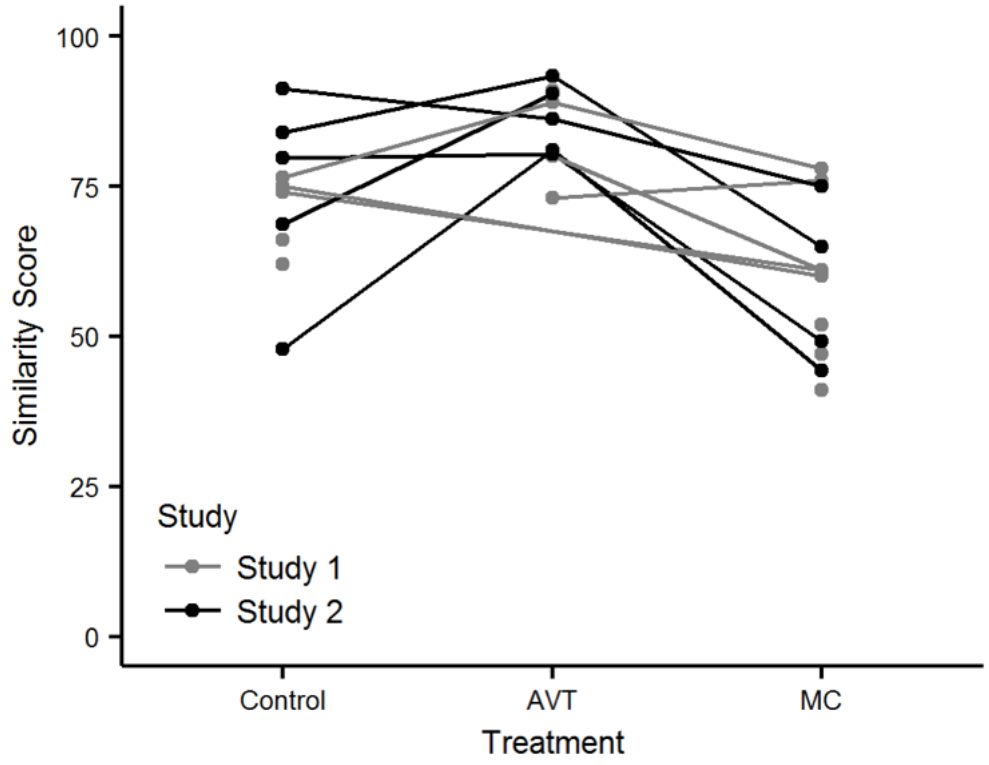

Figure 5.4. Individual similarity scores at day 90 for both Study 1 and Study 2.
Lines connect siblings within the same family. The similarity score for subjects in Study 1 is from a single song recording, whereas the mean similarity score is shown for subjects in Study 2.

b) Social Motivation and Attention to Social Cues Influences Song Learning.

(i) Study 1

Song similarity was found to correlate with a number of measures of social motivation throughout development. There was a negative correlation between similarity and the number of perch hops (activity level) when newly-fledged subjects were isolated from their parents and family (Figure 5.5A, $X^2(1) = 5.9$, $p = 0.015$). Increased activity during isolation is indicative of atypical social development; fledgling zebra finches typically remain silent and motionless when left alone during

parental foraging bouts (Zann, 1996). In addition, we found that the time spent in proximity to the parents in the four-way test of affiliative preferences at day 30 was positively correlated with similarity score (Figure 5.5B, $X^2(1) = 5.3$, $p = 0.021$). Additionally, increased time spent in proximity to any other birds (adult males, adult females, or parents) at both days 72 and 86 post-hatch was associated with higher similarity scores (Figure 5.5C and 5.5D, Day 72, $X^2(1) = 5.7$, $p = 0.017$; Day 79, $X^2(1) = 2.6$, $p = 0.106$; Day 86, $X^2(1) = 7.2$, $p = 0.0074$).

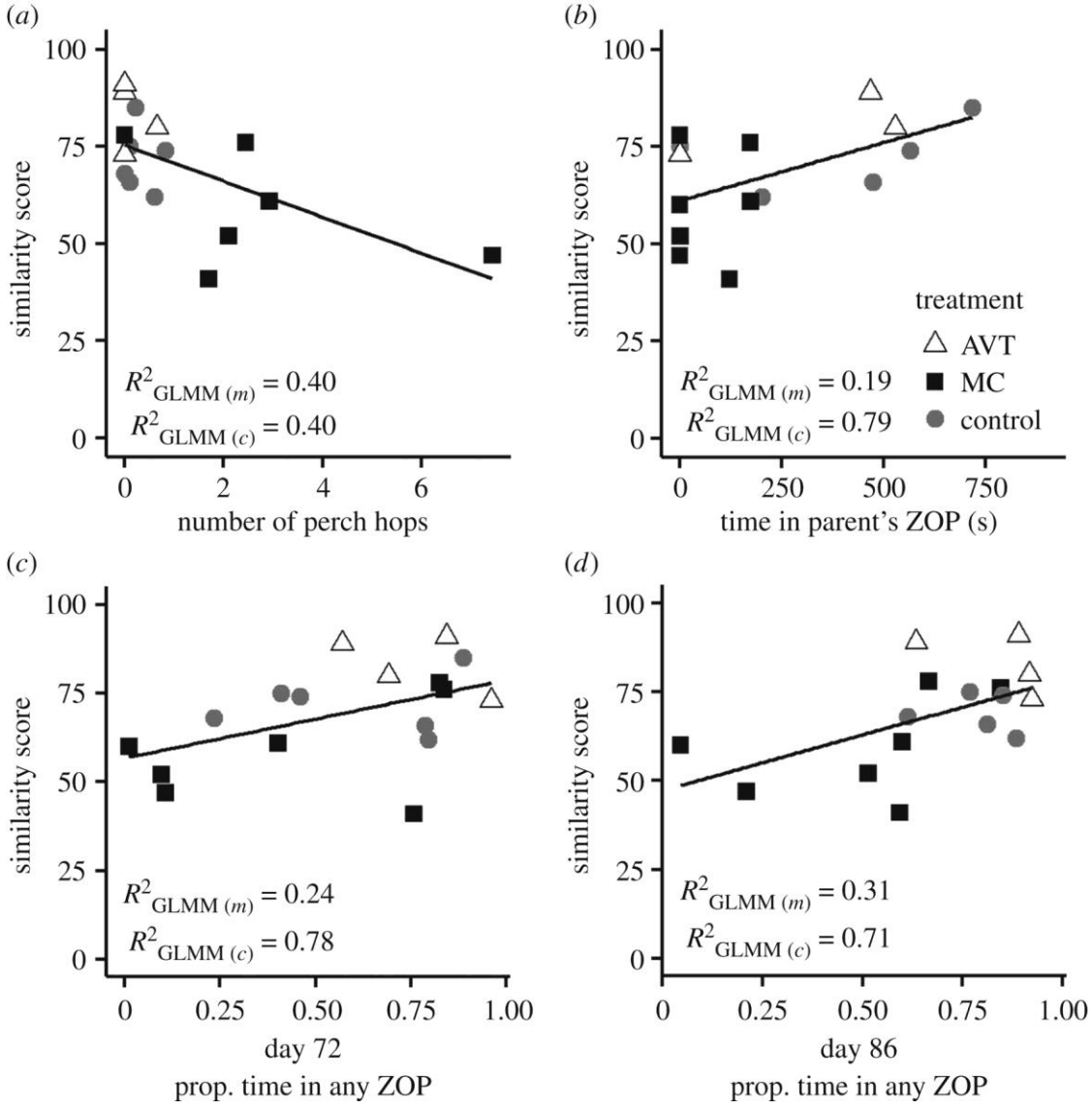

Figure 5.5. Scatterplots of the similarity scores at 90 dph correlated with juvenile behaviors.

(A) Similarity score negatively correlated with number of perch hops during isolation in the social isolation test. (B) Similarity score positively correlated with time in seconds spent in the zone of proximity (ZOP) with the male and female parent during the four-way affiliative preference test on 30 dph, and the proportion of total test time spent in any of the three ZOPs during the four-way affiliative preference tests on (C) 72 dph and (D) 86 dph. The lines depict significant general linear model fits.

(ii) Study 2

Several acoustic features of the songs differed between treatment groups, including amplitude, Weiner entropy, pitch, and harmonic structure (Figure 5.6, Table 5.1). However, there was no evidence that our manipulation caused motor impairment, as acoustic measures of the songs produced by manipulated birds fell within normal ranges for zebra finches (Wood et al., 2013; Riede, Schilling & Goller, 2012). Additionally, we explored the possibility that nonapeptide treatment either accelerated or delayed the time for song to reach its stable mature form. Treatment groups did not differ in any measure of the amount of singing during the seven recording sessions between 50 and 80 dph (i.e. latency to sing, amount of singing, number of days in which singing occurred, or earliest date of singing). Furthermore, there was no effect of treatment on individual variability across song bouts in any treatment group at day 120 (Similarity, $X^2(2) = 0.37$, $p = 0.83$; Accuracy, $X^2(2) = 0.59$, $p = 0.75$), suggesting treatment did not impact the timing of song crystallization.

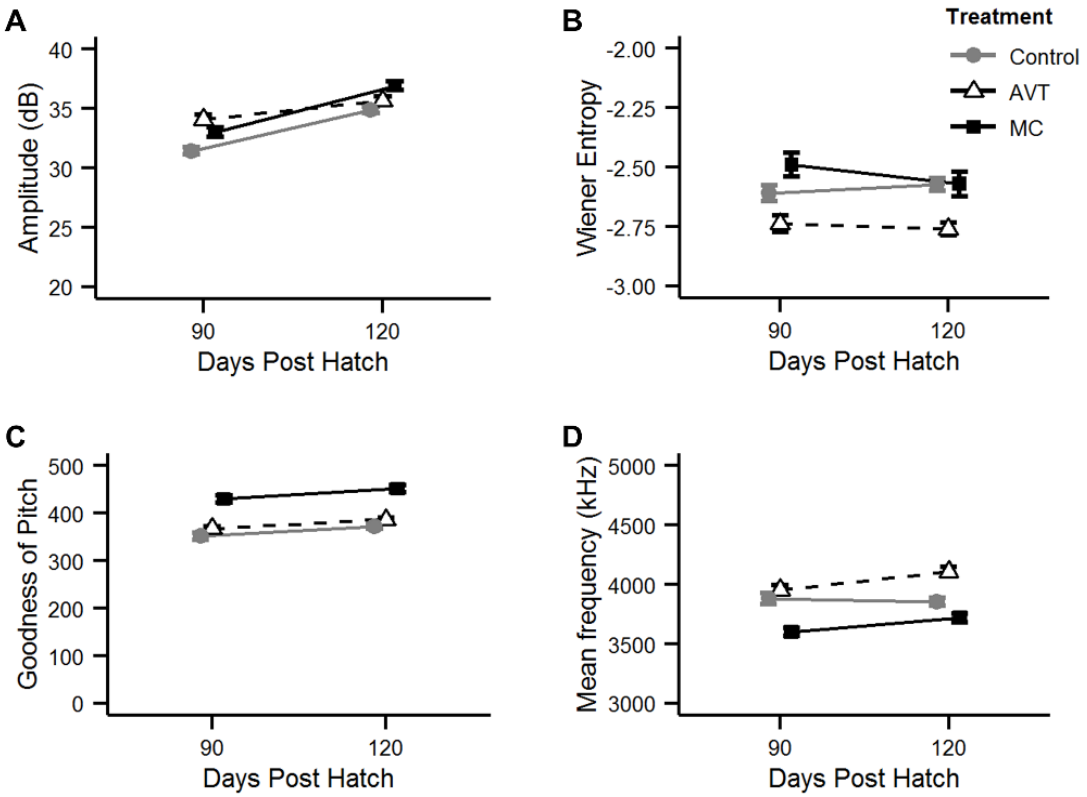

Figure 5.6. Acoustic features of song recorded on 90 and 120 days post-hatch.
Mean ± SE of (A) Amplitude (dB) of the song motif. All groups became louder between 90 dph and 120 dph (t =10.1, p < 0.0001). AVT birds were louder on 90 dph (t = 3.0, p = 0.01), but decreased less in loudness between 90 dph and 120 dph than both MC and Control males (Control-AVT: $X^2(1) = 14.4$, p = 0.0003; AVT-MC: $X^2(1) = 16.2$, p = 0.0002). (B) Wiener entropy as measured using Sound Analysis Pro (SAP). Wiener entropy slightly but significantly increased in the Control group between 90 dph and 120 dph (t = 2.5, p = 0.02), but it decreased in both the AVT and MC males (AVT: t = -2.4, p = 0.03; MC: t = -3.2, p = 0.004). (C) Goodness of pitch. The goodness of pitch actually increased between 90 dph and 120 dph (t = 5.4, p = 0.0002). However, MC birds had a higher goodness of pitch than Control males (t = 2.6, p = 0.02). (D) Mean frequency (kHz). MC males had a lower mean frequency compared to Controls (t = -2.8, p = 0.009). Additionally, both AVT and MC males, but not Controls, increased in mean frequency between 90 dph and 120 dph (AVT: t = 2.7, p = 0.01; MC: t = 2.2, p = 0.04). Control subjects are depicted with circles and a solid gray line, AVT with triangles and dashed black line, and MC as squares and solid black line.

Thus, we sought to determine the factors that led to the differences in similarity across treatments by comparing numbers of tutor syllables copied in each group (Figure 5.1). We found that MC males copied fewer syllables from their tutor

than Control and AVT males (55% versus 88% and 87%, respectively) ($X^2(2) = 6.42$, $p = 0.04$; Control-AVT: $Z = 0.91$, $p = 0.76$; Control-MC: $Z = 5.72$, $p = 0.03$; and AVT-MC: $Z = 7.17$, $p = 0.02$). However, the treatment groups did not differ in either the similarity or accuracy of individual syllables (Similarity, $X^2(2) = 1.26$, $p = 0.53$; Accuracy, $X^2(2) = 0.18$, $p = 0.91$). However, the lower accuracy of the whole song among MC males in Study 1 suggests that the accuracy of individual syllables may have been affected in this study. Surprisingly, AVT males also did not differ from Controls on any individual feature of acoustic similarity to the tutor's song. This indicates that AVT males' improved similarity score was a result of additive effects of multiple slight improvements in Wiener entropy, spectral continuity, pitch, and frequency modulation, from which the similarity score is calculated (Tchernichovski et al., 2000).

Table 5.1. Linear mixed model (LMM) results for acoustic features of song.
Summary of the linear mixed models testing for an interaction effect between treatment and day (90 dph vs 120 dph) on individual acoustic features (Duration ($X^2(2) = 8.3$, $p = 0.02$), Amplitude ($X^2(2) = 19.9$, $p < 0.0001$), Pitch ($X^2(2) = 7.8$, $p = 0.02$), Mean Frequency ($X^2(2) = 8.0$, $p = 0.02$), Peak Frequency ($X^2(2) = 8.7$, $p = 0.01$), Goodness of Pitch ($X^2(2) = 5.7$, $p = 0.06$), Wiener Entropy ($X^2(2) = 11.1$, $p = 0.004$), Frequency Modulation ($X^2(2) = 26.4$, $p < 0.0001$), and Amplitude Modulation($X^2(2) = 9.0$, $p = 0.01$)) as dependent variables. The fixed effects are Treatment, Day Post-Hatch (dph), and the interactions. Individual ID nested within Family ID was included as a random effect. The LMM models were selected based on model comparisons using likelihood ratio tests. To test the significance of each parameter within the models, we used the Kenward-Roger approximation to get approximate degrees of freedom and the t-distribution (SE = standard error, bold numbers indicate significance, * refers to an interaction term).

	Duration(s)				*Amplitude (dB)*				*Pitch*			
Predictors	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*
Intercept	737.249	88.109	8.367	**0.000**	31.169	0.987	31.585	**0.000**	1186.953	93.270	12.726	**0.000**
DPH (120)	-52.210	16.470	-3.170	**0.005**	3.715	0.366	10.142	**0.000**	27.173	12.717	2.137	**0.048**
Treatment (AVT)	-104.705	124.546	-0.841	0.410	3.187	1.079	2.954	**0.011**	124.776	94.786	1.316	0.207
Treatment (MC)	90.143	130.548	0.690	0.497	1.801	1.141	1.578	0.139	-197.378	100.791	-1.958	0.068
Treatment (AVT)* DPH(120)	51.562	23.296	2.213	**0.038**	-1.968	0.518	-3.798	**0.002**				
Treatment (MC)*DPH(120)	-12.670	23.676	-0.535	0.598	0.152	0.527	0.288	0.778				

	Mean Frequency (kHz)				*Peak Frequency (kHz)*				*Goodness of Pitch*			
	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*
Intercept	3861.651	63.142	61.158	**0.00E+00**	3839.487	70.819	54.216	**0.00E+00**	352.743	23.606	14.943	**1.50E-08**
DPH (120)	-5.069	38.757	-0.131	0.897	-11.629	43.921	-0.265	0.793	19.905	3.700	5.380	**0.000**
Treatment (AVT)	93.177	88.839	1.049	0.303	105.937	99.628	1.063	0.297	22.449	25.784	0.871	0.403
Treatment (MC)	-258.672	92.597	-2.794	**0.009**	-278.558	103.830	-2.683	**0.012**	71.680	27.366	2.619	**0.024**
Treatment (AVT)* DPH(120)	145.944	54.795	2.663	**0.013**	179.942	62.094	2.898	**0.007**				
Treatment (MC)*DPH(120)	121.286	55.746	2.176	**0.038**	127.605	63.173	2.020	0.053				

	Wiener Entropy				*Frequency Modulation*				*Amplitude Modulation*			
	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*
Intercept	-2.667	0.134	-19.883	**2.15E-14**	39.109	1.529	25.571	**1.67E-11**	-0.010	0.001	-8.882	**1.07E-10**
DPH (120)	0.094	0.038	2.459	**0.023**	1.075	0.368	2.923	**0.013**	0.002	0.001	3.077	**0.004**
Treatment (AVT)	-0.060	0.156	-0.385	0.704	2.095	1.439	1.455	0.172	-0.001	0.002	-0.862	0.394
Treatment (MC)	0.163	0.165	0.989	0.335	1.652	1.530	1.079	0.302	-0.002	0.002	-1.036	0.307
Treatment (AVT)* DPH(120)	-0.130	0.054	-2.408	**0.026**	-1.502	0.520	-2.888	**0.014**	-0.003	0.001	-3.012	**0.005**
Treatment (MC)*DPH(120)	-0.177	0.055	-3.227	**0.004**	-2.769	0.529	-5.237	**2.39E-04**	-0.001	0.001	-1.278	0.209

Discussion

To our knowledge, these are the first findings demonstrating the effects of early life manipulations of nonapeptides in a species that exhibits vocal learning. Our studies provide several converging lines of evidence suggesting that song learning outcomes were impacted by treatment-mediated changes to social motivation during development. First, we found several significant correlations between song learning and measures of social affiliation to both parents and conspecifics during development. Second, MC males only learned portions of their tutors' song, but did not differ in the acoustic match of individual syllables, suggesting that treatment effects were not driven by generalized effects on motor capacity. MC males copied fewer tutor syllables and several exhibited abnormal repeated notes characteristic of isolate-reared song at the beginning of their core motif (Williams, 2004). Third, AVT males in Study 2 exhibited improved skill in matching multiple features of their song to those of their tutors. In Study 1, early life nonapeptide treatment was found to alter a whole suite of motivated social behaviors. MC males exhibited less—and AVT males more—affiliative interest in their parents throughout development (Baran, Sklar & Adkins-Regan, 2016). Furthermore, although manipulated birds had longer latencies to sing to females (Figure 5.7), AVT males were several orders of magnitude more affiliative with their partner than both MC and Control males (Baran, Tomaszycki & Adkins-Regan, 2016).

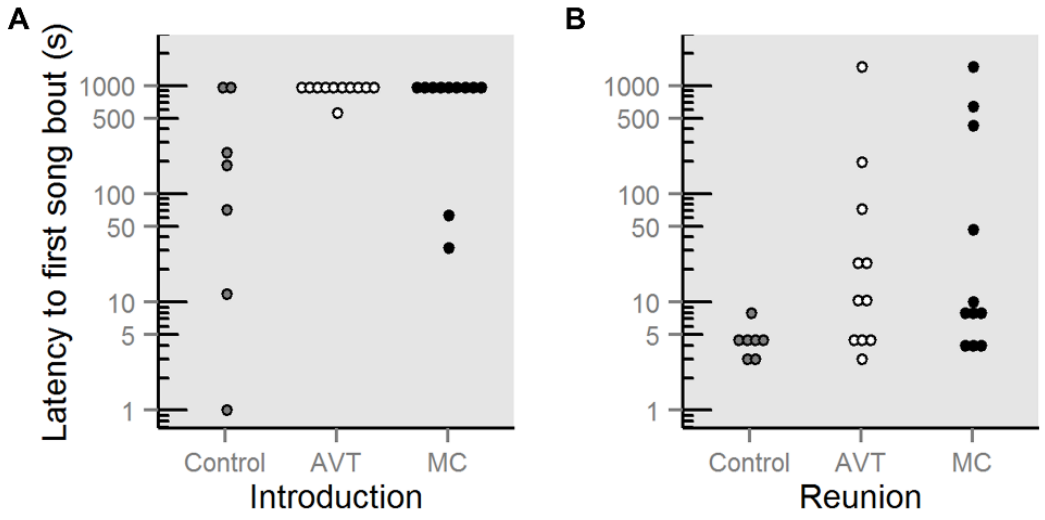

Figure 5.7. Latency to sing when introduced to and later reunited with a female partner. (A) Dot plot of the latency (s) until the male's first song bout during introduction to a novel female conspecific ("partner"). AVT and MC males took significantly longer to sing than Controls when first introduced to a novel female (Kruskal-Wallis test: $X^2(2) = 9.26$, p = 0.01; AVT-Control: p = 0.005, MC-Control: p = 0.05; AVT-MC: p = 0.5) (B) Latency (s) during reunion with the female partner following a one-hour separation after being housed with that female for one week. AVT and MC males also took significantly longer to sing upon reunion (Kruskal-Wallis test: $X^2(2) = 7.1$, p = 0.03; AVT-Control: p = 0.02, MC-Control: p = 0.02; AVT-MC: p = 0.9). Latency to first song bout (s) is shown on a $\log_{10}$-scale.

Our neuroendocrine manipulation, conducted very early in development, demonstrated that a relatively non-localized intracranial administration of AVT or MC could create long-lasting effects. Since the changes to social and vocal behavior were observed over development, long after the injections were performed, our findings were not due to immediate activational effects on the AVT system during the first week of life. Instead, these results suggest that AVT plays an organizational role in the development of social and vocal circuits. Receptors for AVT, including V1aR and OTR, are widespread throughout the male zebra finch brain in adulthood. However, we know little about the development of the AVT system in songbirds, and there is limited evidence that the classic song learning anterior forebrain pathway (AFP) is

directly modulated by AVT (Leung et al., 2009; Voorhuis & De Kloet, 1992; Leung et al., 2011; Kimura, Okanoya & Wada, 1999). Thus, our findings suggest that other nonapeptide-sensitive sensorimotor and socio-motivational brain regions must be an important part of the vocal learning pathway (Rose and Moore, 2002; Syal and Finlay, 2011). Both AVT and V1aR appear to be involved in both sensory and motor components of vocal behavior in adult songbirds. Several structures in the auditory forebrain, including the caudomedial mesopallium (CMM) and the caudomedial nidopallium (NCM), highly express V1aR in zebra finches (Leung et al., 2011). In addition to limited expression within RA, two nuclei involved in the motor pathway of song production contain high densities of AVT receptors: the intercollicular nucleus (ICo, a region implicated in vocal control) and nXIIts (the motor nucleus which innervates the syrinx) in several species (Leung et al., 2009; Voorhuis & De Kloet, 1992; Leung et al., 2011; Kiss et al., 1987; Panzica et al., 1999).

In addition, our data suggest that changes to social motivation impact song learning outcomes, and that nonapeptides acting in the highly-conserved mesolimbic reward and social behavior networks provide a plausible neurobiological mechanism (Goodson, 2005; O'Connell and Hofmann, 2011). Numerous studies in other species provide evidence that nonapeptides may play an important role in experience-dependent development of social behavior (Hammock, Law & Levitt, 2013; Boer et al., 1994; Winslow & Insel, 1993; Veenema, Bredewold & De Vries, 2012; Veenema, Bredewold & De Vries, 2013; Bredewold et al., 2014; Bales & Carter, 2003a; Bales & Carter, 2003b; Bales et al., 2004; Bales et al., 2007; Yamamoto et al., 2004; Yamamoto, Carter & Cushing, 2006; Mogi et al., 2014). In zebra finches, AVT-

immunoreactive fibers and V1aR are densely expressed in the ventral tegmental area (VTA), a region central in reward, motivation, and reinforcement learning circuits (Leung et al., 2009; Voorhuis & De Kloet, 1992; Leung et al., 2011; Gale and Perkel, 2010). AVT cell groups in the medial amygdala and medial bed nucleus of the stria terminalis (BSTm) send substantial projections to VTA. The subsequent connections between VTA and the nucleus accumbens (NAcc) form an important part of the mesolimbic reward pathway, which modulates the behavioral responses to rewarding or motivating stimuli. Our previous research showed that males treated with AVT have altered expression of V1aR and immediate early gene activity in the medial amygdala and BSTm, suggesting treatment changed the activity of this pathway (Baran, Tomaszycki & Adkins-Regan, 2016).

The VTA also projects to the song learning system via dopaminergic input to the striatal Area X, innervating this nucleus most strongly during socially-motivated singing (Kubikova & Košťál, 2010). Furthermore, the activity of dopaminergic cells in the VTA during song learning from a social partner is associated with better learning outcomes (Chen, Matheson & Sakata, 2016; Gadagkar et al., 2016). Thus, the pathway connecting the VTA to the AFP may allow for motivational modulation of song learning.

These studies provide a plausible neurobiological foundation for links between social motivation and song learning systems as an explanation for our findings. However, our data do not allow us to rule out the possibility that alterations to early vocal or social behavior, rather than long-term organizational effects of nonapeptides on the brain, resulted in the observed changes to song development. We targeted our

manipulation to alter the species-typical trajectory of the AVT system early in development, which likely resulted in widespread changes to multiple brain systems important in social function, including the hypothalamic-pituitary adrenal (HPA) axis, sensorimotor systems, and socio-motivational systems. For example, it is possible that treatment stimulated vocalization at the time of treatment, facilitating later vocal motor behavior. However, zebra finches do not make vocalizations at all until at least 3 dph and then they may or may not vocalize during feeding from 4-12 dph (Perez et al., 2016). It is also possible that, by altering interactions between parents and offspring at the time of treatment, these early life manipulations of the AVT system could have resulted in a self-reinforcing feedback loop in parent-offspring social interactions, leading to lifelong changes to social behavior. For example, early life manipulations of the HPA axis via corticosterone administration in wild zebra finch chicks alters begging vocalizations, which in turn alters parental feeding behavior (Perez et al., 2016). Thus, AVT manipulations may alter early vocalizations or interactions between parents and offspring in the nest.

Conclusion

A major innovation necessary for the evolution of vocal learning is thought to be the linkage between the neural representation of social partners, motivational circuitry, and communicative systems (Syal & Finlay, 2011). Consistent with this idea, we propose that nonapeptide treatment has altered males' motivation to attend to socially-relevant cues, or has changed the salience of those cues, during vocal learning. The reduced similarity in MC males' song may result from reduced attention

or sensitivity to behavioral feedback from tutors, whereas AVT males were more motivated to affiliate with and attend to social partners.

Our findings indicate that the developing brains of songbirds are modulated by nonapeptides in ways that are crucial for communicative development. Nonapeptides are known to play a role in diverse physiological functions including the stress response via regulation of the hypothalamic-pituitary-adrenal axis, sensorimotor processes, and social behaviors—all of which may impact the process of vocal learning. Despite well-known associations between social deficits and language impairments, the present study is among the first to assess the effect of developmental exposure to AVT/AVP on a vocal learner of any species. Given the strong parallels between songbird and human vocal learning at multiple levels of organization (Doupe & Kuhl, 1999) nonapeptides likely play a similarly important role in the communicative development of humans. Further investigation is urgently needed, as exogenous administration of nonapeptides is currently being tested in clinical trials in children diagnosed with autism spectrum disorder, a developmental disorder associated with deficits in social motivation (Warlaumont et al., 2014; Chevallier et al., 2012). Our findings open the door for further work on the neural and neuroendocrine mechanisms underlying social and communicative development.

Methods

Study 1: Organizational Effects of Nonapeptides on Social Development and Song Quality

Seventy-two unpaired adult males and females bred in six large aviaries (1.2 x 0.9 x 0.6 m). Male offspring hatched within 40 days were used as experimental subjects. Starting on day 2 post-hatch through day 8, subjects received daily 2 µL IC injections of either 1) AVT (10ng); 2) Manning Compound (50ng); or 3) 0.9% isotonic saline vehicle control (Goodson, Lindberg & Johnson, 2004; Manning et al., 2012; Baran, Sklar & Adkins-Regan, 2016). This time point was chosen to target an important period of growth and maturation of the avian medial amygdala and, specifically, the predicted development of AVT neurons in both hypothalamic and medial amygdala structures (Ikebuchi et al., 2013; Buijs, Velis & Swaab, 1980; Szot & Dorsa, 1993). Injections targeting more specific brain regions are not possible at this point in development because this is a major period of neurogenesis in the zebra finch brain. The forebrain, in particular, is increasing in volume by several orders of magnitude between days 2-8 post-hatch (Ikebuchi et al., 2013; Charvet & Striedter, 2009). We followed the intracranial injection methodology detailed in (Baran, Sklar & Adkins-Regan, 2016; Bender & Veney, 2008). Following injection, we verified that chicks exhibited normal begging behavior (mouth gaping) in response to tactile stimulation before returning them to the nest. No behavioral data were collected from the chicks during the treatment period or prior to fledging. Both AVT and MC act at multiple receptor subtypes in the zebra finch brain, including the VT4 (V1aR), VT3 (OT-like), and V2 receptors (Manning et al., 2012; Leung et al., 2009).

In order to study song development in a naturalistic social setting, subjects were cared for by the parents until approximately 40 days of age, when zebra finches normally become independent of parental feeding. After 39.8 ± 5.4 days, subjects were housed in same-sex aviaries in a separate room from the parents. The only interactions between subjects and their parents after moving into same sex housing occurred during the weekly 15 minute four-way affiliative preference tests which took place from 30 dph to 86 dph (detailed below) (Baran, Sklar & Adkins-Regan, 2016).

To understand the effect of treatment on social motivation throughout development, we measured responsiveness to isolation from the family and subsequent reunion with the male parent the day after fledging. The day after subjects from Study 1 only were first observed having fledged from the nest, we assessed subjects' responses to isolation from their family and subsequent reunion with the male parent. The social isolation tests were performed in a testing apparatus (60 x 41 x 36 cm) in a separate room from the breeding cage. Two aviaries of paired adults were in the room but were behind a curtain to provide ambient colony noise. After one minute of acclimation, we recorded behavior in isolation for 9 min total. Next, the male parent was placed in the aviary with the fledgling for five additional minutes. The video was scored for the number of perch hops, saccadic head movements, and long tonal calls performed by the subject per minute.

In addition, we assessed the changes in affiliation with the parents, unfamiliar males, and unfamiliar females each week throughout juvenile development using a four-way affiliative preference test (Baran, Sklar & Adkins-Regan, 2016). We assessed the Study 1 subjects' preference for being proximal to parents or unfamiliar

conspecifics weekly from day 30 to 86 in four-choice proximity tests with two males, two females, the parent pair, or no conspecifics as the four stimulus choices, similar to (Adkins-Regan and Leung, 2006). For testing, the subject was removed from its aviary and placed alone in a plus-shaped testing cage (61 x 61 x 41 cm) in a separate testing room, which was flanked on three sides by cages containing pairs of stimulus birds. The three stimulus cages (61 x 36 x 45 cm) were positioned next to the subject's cage. One stimulus cage contained the subject's parents, one contained two unfamiliar adult females, and one contained two unfamiliar adult males. Subjects were allowed to acclimate in the apparatus for one minute prior to recording. Tests were 15 minutes long and were videotaped from behind a blind with no human in the room. The testing cage contained three stimulus zones of proximity (ZOPs). The remainder of the cage (the center portion and the zone nearest to the video camera which was not proximal to any birds) was considered a neutral (non-proximity) zone. The same pool of 20 males and 20 females was used as conspecific stimuli in a random order for each subject. The stimuli were unfamiliar to the subject at the time of presentation, with the location of each stimulus set varied randomly. The total time that the subject's head was in each of the three proximity zones was recorded. Proximity is a valid indicator of family and sexual and pairing interest in this species, because these relationships are marked by close physical proximity (Clayton, 1990). The testing period, with nine weekly tests, covered the majority of the juvenile period, allowing us to measure changes in affiliative preferences across juvenile development. All tests were recorded with a Canon Vixia HFM31 HD camera. Digital videos were coded by trained assistants who were blind to treatment. In addition, all researchers were blinded to treatment

throughout the experiment, until after data collection and video coding was complete.

Results from this cohort showed widespread effects of nonapeptide treatment on social development in both male and female juveniles and on pairing behavior and neural activity in adult males (and were previously published in Baran, Sklar & Adkins-Regan, 2016 and Baran, Tomaszycki & Adkins-Regan, 2016). Here we present new results, including song learning analyses and novel correlations, between measures of social motivation and song learning outcomes.

After reaching sexual maturity, subjects were randomly assigned an unmanipulated, sexually-naïve, and unpaired female pair partner. In order to obtain high-quality recordings of the male subjects' songs, all introductions between the subjects and their pair partner were performed on 90 dph in a room with no other birds, in a small aviary (57 x 32 x 42 cm) enclosed by sound attenuating foam. The subject was first placed in the cage, followed by the partner. Behavior (song latency, number of song bouts) was scored for the 15 min following introduction, though pair was sometimes left in the cage for 20-45 min if a male did not sing during the first 15 min. After the introductions, the pair was moved into a small pair aviary (.57 x .32 x .42 m or .61 x .36 x .43 m) in a colony room. The pairing aviaries were arranged such that they were visually, but not acoustically, isolated from other pairs in the room. Subjects were then housed with the partner for a total of seven days. High-quality songs were recorded from social fathers and other adult males in the breeding aviaries using a similar method for comparisons to juvenile songs. All songs were recorded using a highly-directional cardioid microphone (Sennheiser ME66). As a result of profound effects of treatment on singing, a large number of males did not sing (AVT:

n = 10/11; MC: n = 9/11; and Control: n = 2/7) during this introduction. Thus, we attempted to record songs from these males in the colony room over the course of the following week. Recordings were obtained either by an observer in the colony room or from recordings of reunion with the partner following a 1hr separation. These song recordings were processed to remove extraneous noise prior to analysis. In the final analysis, we obtained high-quality song recordings from n = 4 AVT males, n = 8 MC males and n = 6 Control males, which was a subset of all males in the experiment. In addition, for all males in the experiment (AVT: n = 11; MC: n = 11; and Control: n = 7), we recorded the latency to the first song bout during the first introduction to the female partner. We also recorded both the latency to sing and the number of song bouts in the reunion after 1 hr separation from the female partner, with whom they had been housed in a small pair aviary for seven days. High-quality songs were recorded using a similar method from social fathers and all other adult males in the breeding aviaries for comparisons to juvenile songs. An experimental timeline for Study 1 is shown in Figure 5.8.

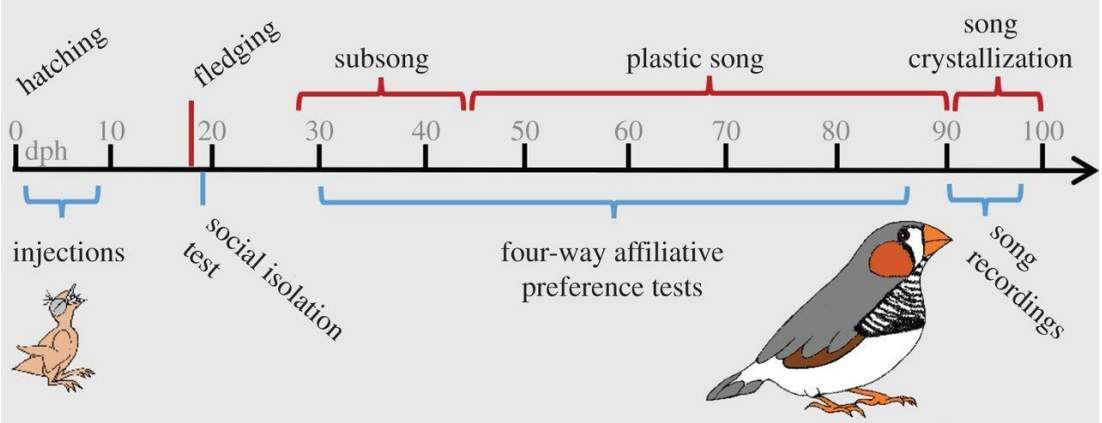

Figure 5.8. Experimental timeline for Study 1.
Developmental events (hatching, fledging, and song milestones) are above the line and experimental events (injections, social isolation tests, and four-way proximity tests, song recordings) are below the line.

Zebra finch males sing a highly-stereotyped song, each comprised of several introductory notes followed by a single repeated motif with stable number of syllables (Williams, 2004). To perform acoustic analysis, a single song motif excluding the introductory notes was randomly selected from subjects' recordings. We assessed song learning using Sound Analysis Pro 2.0 (SAP) to compare the acoustic features of juvenile and tutor song (Tchernichovski & Mitra, 2002). Our analysis focused primarily on the scores of song similarity, accuracy, and sequential match percentage (Tchernichovski et al., 2000). The motifs of each juvenile's song were compared to the respective tutor motif, using the same tutor motif for all of a juvenile's analyses. For all song analyses, we used linear mixed models to analyze the effect of treatment, with family included as a random effect. This allowed us to control for unobserved heterogeneity resulting from individual tutor song or family effects.

Study 2: Organizational Effects of Nonapeptides on Vocal Learning

In the second experiment, we used cross-fostering on the second day post-hatch to create a within-family design to control for tutor and genetic factors which likely influence song learning. The genetic sex of the subject birds was determined on the day of hatching and chicks were then cross fostered on 2 dph to create families with three male subjects (one per treatment group) and one non-subject female sibling. All experimental subjects and female siblings were raised by foster parents until 60 dph. The experiment was conducted using four temporally separate family cohorts (n = 7 families), each with a total clutch size of four. However, two families in the same aviary were excluded from analyses because one of the male parents was highly aggressive towards his own offspring (two of which died), his female partner (who also died), and the other juveniles within the aviary, resulting in a highly abnormal developmental environment for all surviving offspring within the aviary. Additionally, one subject was excluded from the study because of incorrect genetic sexing. Any additional females were cross-fostered into nests in a non-experimental aviary to be raised for future studies. Thus, the final number of subjects at the end the experiment totaled 14, consisting of AVT (n = 5), Manning Compound (n = 4), and saline control (n = 5) subjects. For both Study 1 and Study 2, birds were kept on a 14/10h light/dark cycle and were provided with seed, cuttlebone, water, and grit ad libitum throughout the study, with supplemental hard-boiled eggs weekly during the egg laying period. Additionally, each aviary was equipped with a nest box and nest-building material (coconut fiber), allowing the parent pairs to construct nests and breed. Nest boxes were checked daily in the morning to record the number of eggs and chicks. On the

day of hatching, subjects were genotyped to determine the genetic sex using DNA extracted from feather follicle tissue (Baran, Sklar & Adkins-Regan, 2016). Chicks were marked with non-toxic markers for individual identification. In both studies, all researchers were blinded to treatment until after the completion of the experiment. All procedures were developed with veterinary supervision and approved by Cornell University's Institutional Animal Care and Use Committee.

Songs were recorded from subjects between 50 and 120 dph. To obtain song recordings at each time point, subject males were removed from their home aviaries and individually isolated in sound attenuation chambers overnight. The chambers were kept in a separate room and were constructed from coolers (0.94 x 0.38 x 0.38 m) internally lined with sound attenuating foam. Individual transport cages (0.46 x 0.23 x 0.25 m) along with a microphone were placed in the chambers. Keeping the juveniles in the room overnight allowed them to become accustomed to the chamber and social isolation increased subsequent motivation to sing. Each chamber was equipped with overhead white LED lights, and subjects were provided with seed, cuttlebone, and water ad libitum while in the chambers. The next day immediately following lights on, a female was introduced to the cage with the subject. Song was recorded every three days from 50 to 60 dph, every ten days from 60 dph until 90 dph, and on 120 dph for 1 hr each day. The female used to elicit song was the same on 90 and 120 dph for each male subject, to control for differing motivation to sing to different females. Male subjects were kept in the sound attenuation chambers for as long as necessary to record multiple song bouts, even when that required several hours of recording. Acoustic analyses followed the procedures of Study 1, but additional song recording

time at day 90 and 120 allowed us to obtain ten motifs from each juvenile on each recording day. Collecting these additional motifs allowed us to perform more detailed song analyses, including analysis of Wiener entropy, pitch, and harmonic structure, as well as syllable-level descriptions of song copying.

Song Recording Analysis

In Study 1, one motif was cropped at random from subjects' recordings. In Study 2, ten motifs were cropped at random from juvenile songs recorded at 90 dph and at 120 dph. Motifs with background noise, female calls, or cage noise were excluded from the sample. Introductory notes were identified and excluded from analysis. A total of four core motifs were cropped out at random from the songs recorded from the focal juvenile's father and the best core motif was chosen based on the least amount of background noise or cage noise. Sound files were saved as uncompressed digital audio to a hard disk with a sampling rate of 44.1 KHz.

As a measure of song learning success, the recordings were used to analyze juvenile song match to paternal song using Sound Analysis Pro 2.0 (SAP) (Tchernichovski et al., 2000). SAP measures song similarity between juvenile and paternal song by splitting up the songs into syllables, defined as discrete sound units bounded by silent intervals. For each tutor-juvenile pair of songs, SAP calculates the probability that the goodness of match between the songs would have occurred by chance (Tchernichovski & Mitra, 2002). Our analysis focused primarily on the scores of song similarity, accuracy, and sequential match percentage. Percent song similarity is defined as the percentage of tutor sounds included in the juvenile's crystallized

song. Tutor/pupil pairs typically have similarity scores between 65 and 95, whereas random pairs typically have scores ranging between 20 and 45 (Tchernichovski et al., 2000). Song accuracy is the average local similarity per millisecond across the crystallized song. Sequential match is calculated by comparing song tempo and rhythm between the tutor song and the juvenile's crystallized song (Tchernichovski et al., 2000). In SAP, the similarity, accuracy, and sequential match were determined using the Explore & Score feature segmentation tool (entropy: -9.5; FFT data window: 9.27 ms; contour threshold: 10; frequency range: 11025 Hz; advance window: 1.00 ms). The motifs of each juvenile's song were compared to the respective father motif, using the same father motif for juvenile day 90 song and day 120 song analyses.

AVT has also been shown to affect acoustic features of vocalizations in other vertebrate species, so we tested whether the treatment impacted the spectral features of the song which have good articulatory correlates. For the Study 2 subject songs, we used Sound Analysis Pro 2.0 (SAP) to analyze each subject's song for the following acoustic features: duration (s), amplitude (dB), pitch, mean frequency (kHz), peak frequency (kHz), Goodness of Pitch, Wiener Entropy, amplitude modulation, and frequency modulation.

In order to investigate individual song variation between motif renditions, and to determine whether inter-group differences in similarity scores were primarily driven by variation in tutor imitation fidelity at the level of the individual syllable or the full motif, we compared all syllables from the chosen tutor motif with all syllables of the ten randomly chosen motifs of each juvenile's crystallized song at 120 dph. For each syllable pair, percent similarity and accuracy were computed. Matching syllables were

identified based on syllable pairs with the highest similarity scores. Syllables for which SAP calculated a similarity score of zero were not included in analysis. Average percent similarity and accuracy of the ten renditions of each syllable and their standard deviations were computed for each juvenile-tutor syllable pair, which was used to calculate overall syllable similarity between pupils and tutors. We then determined the overall percentage of syllables learned from the tutor based on the number of syllables produced by the juvenile which had a >40% similarity score with the equivalent tutor syllable.

Statistical Analyses

All statistical analyses were performed with R software (R Development Core Team 2007). We used random slope linear mixed models (LMM) to test the effect of the treatment on the acoustic features of the song and measures of similarity to the social father. We used the lmer function of the lme4 package (Bates et al., 2014) which allowed us to define multiple distinct random factors. In these models, Treatment (and song recording date in Study 2) were specified as fixed factors. The interaction effect considered was Treatment*Day (in Study 2 only). Random factors were individual ID (18 levels in Study 1 and 17 levels in Study 2), nested within Family ID (13 levels in Study 1 and 6 levels in Study 2). To test the effect of the treatment on the syllable-by-syllable similarity and accuracy scores, we used a random slope LMMs. Random factors were individual ID (17 levels), nested within Family ID (6 levels). To test the effect of treatment on the number of number of notes copied from the tutor, we computed a measure of the proportion of notes copied by dividing

the number of comparable notes by the total number of notes in the tutor's song. We then performed a random slope LMM similar to above with Family ID as a random factor. To perform model comparisons for the LMM models, we used likelihood ratio tests to compare the full model to a reduced null model with only the factor of interest removed using the anova function to perform a chi-square test. To test the significance of each fixed effect within a model, we used the Kenward-Roger approximation to get approximate degrees of freedom and the t-distribution to get p-values (Kenward-Roger in the pbkrtest package) (Højsgaard, 2014). In addition, we performed post hoc tests on the interaction terms using the testInteractions function in the phia package (Rosario-Martinez, Fox & Team, 2015). We also calculated marginal and conditional R2 to measure effect size for the model using the r.squaredGLMM function in the MuMIn package (Bartoń, 2015; Johnson, 2014; Nakagawa & Schielzeth, 2013).

In order to test for treatment effects on whether or not males sang when introduced to a female, we used a chisquare test. To test the effect of IC injections on song latency (which were not normally distributed), we used a nonparametric Kruskal-Wallis test, followed by a pair-wise Wilcoxon test to perform planned comparisons between the different treatment groups.

Ethics

All procedures were developed with veterinary supervision and approved by Cornell University's Institutional Animal Care and Use Committee (Protocol # 2011-0130).

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REFERENCES

Adkins-Regan, E. & Leung, C. H. (2006). Sex steroids modulate changes in social and sexual preference during juvenile development in zebra finches. *Hormones and Behavior, 50,* 772–778. (doi:10.1016/j.yhbeh.2006.07.003)

Adret, P. (2003). Vocal imitation in blindfolded zebra finches (Taeniopygia guttata) is facilitated in the presence of a non-singing conspecific female. *Journal of Ethology, 22,* 29–35. (doi:10.1007/s10164-003-0094-y)

Bales, K.L. & Carter, C.S. (2003a). Developmental exposure to oxytocin facilitates partner preferences in male prairie voles (Microtus ochrogaster). *Behavioral Neuroscience, 117*, 854–859. (doi:10.1037/0735-7044.117.4.854)

Bales, K.L. & Carter, C.S. (2003b). Sex differences and developmental effects of oxytocin on aggression and social behavior in prairie voles (Microtus ochrogaster). *Hormones and Behavior, 44*, 178–184. (doi:10.1016/S0018-506X(03)00154-5)

Bales, K.L., Abdelnabi, M., Cushing, B.S., Ottinger, M.A., & Carter, C.S. (2004). Effects of neonatal oxytocin manipulations on male reproductive potential in prairie voles. *Physiology & Behavior, 81*, 519–526. (doi:10.1016/j.physbeh.2004.02.016)

Bales, K.L., Plotsky, P.M., Young, L.J., Lim, M.M., Grotte, N., Ferrer, E., & Carter, C.S. (2007). Neonatal oxytocin manipulations have long-lasting, sexually dimorphic effects on vasopressin receptors. *Neuroscience, 144*, 38–45. (doi:10.1016/j.neuroscience.2006.09.009)

Baran, N.M., Sklar, N.C., & Adkins-Regan, E. (2016). Developmental effects of vasotocin and nonapeptide receptors on early social attachment and affiliative behavior in the zebra finch. *Hormones and Behavior, 78,* 20–31. (doi:10.1016/j.yhbeh.2015.10.005)

Baran, N.M., Tomaszycki, M.L., & Adkins-Regan, E. (2016). Early life manipulations of the nonapeptide system alter pair maintenance behaviors and neural activity in adult male zebra finches. *Frontiers in Behavioral Neuroscience, 58*. (doi:10.3389/fnbeh.2016.00058)

Bartoń, K. (2015). *MuMIn: Multi-Model Inference.*

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H. & Dai, B. (2014). *lme4: Linear mixed-effects models using Eigen and S4*. [cited 2015 Jan. 21].

Bender, A.T, Veney, S.L. (2008). Treatment with the specific estrogen receptor antagonist ICI 182,780 demasculinizes neuron soma size in the developing zebra finch brain. *Brain Research, 1246*, 47–53. (doi:10.1016/j.brainres.2008.09.089)

Boer, G.J., Quak, J., de Vries, M.C., & Heinsbroek, R.P.W. (1994). Mild sustained effects of neonatal vasopressin and oxytocin treatment on brain growth and behavior of the rat. *Peptides, 15*, 229–236. (doi:10.1016/0196-9781(94)90007-8)

Böhner, J. (1983). Song learning in the zebra finch (Taeniopygia guttata): Selectivity in the choice of a tutor and accuracy of song copies. *Animal Behaviour, 31*, 231–237. (doi:10.1016/S0003-3472(83)80193-6)

Boyd, S.K. (2013). Vasotocin modulation of social behaviors in amphibians. In *Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior* (eds E Choleris, DW Pfaff, M Kavaliers), pp. 97–109. Cambridge, U.K.: Cambridge University Press.

Bredewold, R., Smith, C.J.W., Dumais, K.M., & Veenema, A.H. (2014). Sex-specific modulation of juvenile social play behavior by vasopressin and oxytocin depends on social context. *Frontiers in Behavioral Neuroscience, 8*, 216. (doi:10.3389/fnbeh.2014.00216)

Buijs, R.M., Velis, D.N., & Swaab, D.F. (1980). Ontogeny of vasopressin and oxytocin in the fetal rat: Early vasopressinergic innervation of the fetal brain. *Peptides, 1*, 315–324. (doi:10.1016/0196-9781(80)90009-1)

Charvet, C.J., Striedter, G.F. (2009). Developmental origins of mosaic brain evolution: Morphometric analysis of the developing zebra finch brain. *Journal of Comparative Neurology, 514*, 203–213. (doi:10.1002/cne.22005)

Chen, Y., Matheson, L.E., & Sakata, J.T. (2016). Mechanisms underlying the social enhancement of vocal learning in songbirds. *Proceedings of the National Academy of Sciences*, 201522306. (doi:10.1073/pnas.1522306113)

Chevallier, C., Kohls, G., Troiani, V., Brodkin, E.S., & Schultz, R.T. (2012). The social motivation theory of autism. *Trends in Cognitive Science, 16*, 231–239.

(doi:10.1016/j.tics.2012.02.007)

Clayton, N.S. (1990). Assortative Mating in Zebra Finch Subspecies, Taeniopygia guttata guttata and T. g. castanotis. *Philosophical Transactions of the Royal Society B, 330*, 351–370. (doi:10.1098/rstb.1990.0205)

Derégnaucourt, S., Poirier, C., Kant, A.V. der, Linden, A.V. der, & Gahr, M. (2013). Comparisons of different methods to train a young zebra finch (Taeniopygia guttata) to learn a song. *Journal of Physiology – Paris, 107*, 210–218. (doi:10.1016/j.jphysparis.2012.08.003)

Doupe, A.J. & Kuhl, PK. (1999). Birdsong and human speech: common themes and mechanisms. *Annual Review of Neuroscience, 22*, 567–631. (doi:10.1146/annurev.neuro.22.1.567)

Eales, L.A. (1989). The influences of visual and vocal interaction on song learning in zebra finches. *Animal Behaviour, 37(3),* 507–508. (doi:10.1016/0003-3472(89)90097-3)

Gadagkar, V., Puzerey, P.A., Chen, R., Baird-Daniel, E., Farhang, A.R., & Goldberg, J.H. (2016). Dopamine neurons encode performance error in singing birds. *Science, 354,* 1278–1282. (doi:10.1126/science.aah6837)

Gale, S.D. & Perkel, D.J. (2010). A basal ganglia pathway drives selective auditory responses in songbird dopaminergic neurons via disinhibition. *Journal of Neuroscience, 30*, 1027–1037. (doi:10.1523/JNEUROSCI.3585-09.2010)

Goldstein, M.H. & West, M.J. (1999). Consistent responses of human mothers to prelinguistic infants: The effect of prelinguistic repertoire size. *Journal of Comparative* Psychology, *113,* 52–58. (doi:10.1037/0735-7036.113.1.52)

Goldstein, M.H. & Schwade, J.A. (2008). Social feedback to infants' babbling facilitates rapid phonological learning. *Psychological Science, 19,* 515–523. (doi:10.1111/j.1467-9280.2008.02117.x)

Goldstein, M.H. & Schwade, J.A. (2009) From Birds to Words. In *Oxford Handbook of Developmental Behavioral Neuroscience* (eds MS Blumberg, JH Freeman, SR Robinson), pp. 708–729. Oxford University Press.

Goldstein, M.H., King, A.P., & West, M.J. (2003). Social interaction shapes babbling: Testing parallels between birdsong and speech. *Proceedings of the National Academy of Science, 100*, 8030–8035. (doi:10.1073/pnas.1332441100)

Goodson, J.L. (1998). Territorial aggression and dawn song are modulated by septal vasotocin and vasoactive intestinal polypeptide in male field sparrows (Spizella pusilla). *Hormones and Behavior, 34*, 67–77. (doi:10.1006/hbeh.1998.1467)

Goodson, J.L. (2005). The vertebrate social behavior network: Evolutionary themes and variations. *Hormones and Behaviour, 48*, 11–22. (doi:10.1016/j.yhbeh.2005.02.003)

Goodson, J.L. & Bass, A.H. (2000a). Vasotocin innervation and modulation of vocal-acoustic circuitry in the teleost Porichthys notatus. *Journal of Comparative Neurology, 422*, 363–379. (doi:10.1002/1096-9861(20000703)422:3<363::AID-CNE4>3.0.CO;2-8)

Goodson, J.L. & Bass, A.H. (2000b). Forebrain peptides modulate sexually polymorphic vocal circuitry. *Nature, 403*, 769–772. (doi:10.1038/35001581)

Goodson, J.L., Lindberg, L., & Johnson, P. (2004). Effects of central vasotocin and mesotocin manipulations on social behavior in male and female zebra finches. *Hormones and Behavior, 45*, 136–143. (doi:10.1016/j.yhbeh.2003.08.006)

Goodson, J.L., Rinaldi, J., & Kelly, A.M. (2009). Vasotocin neurons in the bed nucleus of the stria terminalis preferentially process social information and exhibit properties that dichotomize courting and non-courting phenotypes. *Hormones and Behavior, 55*, 197–202. (doi:10.1016/j.yhbeh.2008.10.007)

Hammock, E.A.D., Law, C.S., & Levitt, P. (2013). Vasopressin eliminates the expression of familiar odor bias in neonatal female mice through V1aR. *Hormones and Behavior, 63*, 352–360. (doi:10.1016/j.yhbeh.2012.12.006)

Harding, C.F., Rowe, S.A. (2003). Vasotocin treatment inhibits courtship in male zebra finches; concomitant androgen treatment inhibits this effect. *Hormones and Behavior, 44*, 413–418. (doi:10.1016/j.yhbeh.2003.06.007)

Højsgaard, U.H.S. (2014). *pbkrtest: Parametric bootstrap and Kenward-Roger-based methods for mixed model comparison*. [cited 2015 Jun. 1].

Ikebuchi, M., Nanbu, S., Okanoya, K., Suzuki, R., & Bischof, H-J. (2013). Very early development of nucleus taeniae of the amygdala. *Brain, Behavior, and Evolution, 81*, 12–26. (doi:10.1159/000342785)

Immelmann, K. (1969). Song development in the zebra finch and other estrildid

finches. *Bird Vocalizations, 61*.

Insel, T.R. (2010). The challenge of translation in social neuroscience: A review of oxytocin, vasopressin, and affiliative behavior. *Neuron 65*, 768–779. (doi:10.1016/j.neuron.2010.03.005)

Insel, T.R. & Young, L.J. (2001). The neurobiology of attachment. *Nature Reviews Neuroscience, 2*, 129–136. (doi:10.1038/35053579)

Johnson, P.C.D. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. *Methods in Ecology and Evolution, 5*, 944–946. (doi:10.1111/2041-210X.12225)

Jones, A.E. & Slater, P.J.B. (1993). Do young male zebra finches prefer to learn songs that are familiar to females with which they are housed? *Animal Behaviour, 46*, 616–617. (doi:10.1006/anbe.1993.1233)

Kimura, T., Okanoya, K., & Wada, M. (1999). Effect of testosterone on the distribution of vasotocin immunoreactivity in the brain of the zebra finch, Taeniopygia guttata castanotis. *Life Sciences, 65*, 1663–1670. (doi:10.1016/S0024-3205(99)00415-4)

Kiss, J.Z., Voorhuis, T.A., Van Eekelen, J.A.M., De Kloet, E.R., & De Wied, D. (1987). Organization of vasotocin-immunoreactive cells and fibers in the canary brain. *Journal of Comparative Neurology, 263*, 347–364.

Kojima, S., Doupe, A.J., & Knudsen, E.I. (2011). Social performance reveals unexpected vocal competency in young songbirds. *Proceedings of the National Acadamy of Science USA, 108*, 1687–1692.

Kubikova, Ľ. & Košťál, Ľ. (2010). Dopaminergic system in birdsong learning and maintenance. *Journal of Chemical Neuroanatomy, 39*, 112–123. (doi:10.1016/j.jchemneu.2009.10.004)

Leung, C.H., Goode, C.T., Young, L.J., & Maney, D.L. (2009). Neural distribution of nonapeptide binding sites in two species of songbird. *Journal of Comparative Neurology, 513*, 197–208. (doi:10.1002/cne.21947)

Leung, C.H., Abebe, D.F., Earp, S.E., Goode, C.T., Grozhik, A.V., Mididoddi, P., & Maney, D.L. (2011). Neural distribution of vasotocin receptor mRNA in two species of songbird. *Endocrinology, 152*, 4865–4881. (doi:10.1210/en.2011-1394)

Lipkind, D., Marcus, G.F., Bemis, D.K., Sasahara, K., Jacoby, N., Takahasi, M., Suzuki, K., Feher, O., Ravbar, P., Okanoya, K., & Tchernichovski, O. (2013). Stepwise acquisition of vocal combinatorial capacity in songbirds and human infants. *Nature 498*, 104–108. (doi:10.1038/nature12173)

Ljubičić, I., Hyland Bruno, J., & Tchernichovski, O. (2016). Social influences on song learning. *Current Opionion in Behavioral Science, 7,* 101–107. (doi:10.1016/j.cobeha.2015.12.006)

Lukas, M. & Wöhr, M. (2015). Endogenous vasopressin, innate anxiety, and the emission of pro-social 50-kHz ultrasonic vocalizations during social play behavior in juvenile rats. *Psychoneuroendocrinology, 56*, 35–44. (doi:10.1016/j.psyneuen.2015.03.005)

Maney, D.L., Goode, C.T., & Wingfield, J.C. (1997). Intraventricular infusion of arginine vasotocin induces singing in a female songbird. *Journal of Neuroendocrinology, 9*, 487–491. (doi:10.1046/j.1365-2826.1997.00635.x)

Manning, M., Misicka, A., Olma, A., Bankowski, K., Stoev, S., Chini, B., Durroux, T., Mouillac, B., Corbani, M., & Guillon, G. (2012). Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics. *Journal of Neuroendocrinology, 24*, 609–628. (doi:10.1111/j.1365-2826.2012.02303.x)

Mogi, K., Ooyama, R., Nagasawa, M., Kikusui, T. (2014). Effects of neonatal oxytocin manipulation on development of social behaviors in mice. *Physiology & Behavior, 133*, 68–75. (doi:10.1016/j.physbeh.2014.05.010)

Nakagawa, S. & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution, 4*, 133–142. (doi:10.1111/j.2041-210x.2012.00261.x)

O'Connell, L.A., Hofmann, H.A. (2011). The vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *Journal of Comparative Neurology, 519*, 3599–3639. (doi:10.1002/cne.22735)

Panzica, G.C., Plumari, L., García-Ojeda, E., & Deviche, P. (1999). Central vasotocin-immunoreactive system in a male passerine bird (Junco hyemalis). *Journal of Comparative Neurology, 409*, 105–117. (doi:10.1002/(SICI)1096-9861(19990621)409:1<105::AID-CNE8>3.0.CO;2-8)

Perez, E.C., Mariette, M.M., Cochard, P., Soulage, C.O., Griffith, S.C., & Vignal. C. (2016). Corticosterone triggers high-pitched nestlings' begging calls and

affects parental behavior in the wild zebra finch. *Behavioral Ecology, 27*, 1665–1675. (doi:10.1093/beheco/arw069)

Price, P.H. (1979). Developmental determinants of structure in zebra finch song. *Journal of Comparative Physiological Psychology, 93*, 260–277. (doi:10.1037/h0077553)

Riede, T., Schilling, N., & Goller, F. (2012). The acoustic effect of vocal tract adjustments in zebra finches. *Journal of Comparative Physiology A, 199*, 57–69. (doi:10.1007/s00359-012-0768-4)

Roper, A., Zann, R. (2006). The onset of song learning and song tutor selection in fledgling zebra finches. *Ethology, 112*, 458–470. (doi:10.1111/j.1439-0310.2005.01169.x)

Rosario-Martinez, H.D., Fox, J. & Team, R.C. (2015). *phia: Post-Hoc Interaction Analysis*. [cited 2015 Jun. 1].

Rose, J.D. & Moore, F.L. (2002). Behavioral neuroendocrinology of vasotocin and vasopressin and the sensorimotor processing hypothesis. *Frontiers in Neuroendocrinology, 23*, 317–341. (doi:10.1016/S0091-3022(02)00004-3)

Scattoni, M.L., McFarlane, H.G., Zhodzishsky, V., Caldwell, H.K., Young, W.S., Ricceri, L., & Crawley, J.N. (2008). Reduced ultrasonic vocalizations in vasopressin 1b knockout mice. *Behavioral Brain Research, 187*, 371–378. (doi:10.1016/j.bbr.2007.09.034)

Slater, P.J.B., Eales, L.A., & Clayton, N.S. (1988). Song learning in zebra finches (Taeniopygia guttata): progress and prospects. *Advances in the Study of Behavior, 18*, 1–34.

Syal, S. & Finlay, B.L. (2011). Thinking outside the cortex: social motivation in the evolution and development of language. *Developmental Science, 14*, 417–430. (doi:10.1111/j.1467-7687.2010.00997.x)

Szot, P. & Dorsa, D.M. (1993). Differential timing and sexual dimorphism in the expression of the vasopressin gene in the developing rat brain. *Developmental Brain Research, 73*, 177–183. (doi:10.1016/0165-3806(93)90136-X)

Tchernichovski, O. & Mitra, P. (2002). Towards quantification of vocal imitation in the zebra finch. *Journal of Comparative Physiology A, 188*, 867–878. (doi:10.1007/s00359-002-0352-4)

Tchernichovski, O., Nottebohm, F., Ho, C.E., Pesaran, B., & Mitra, P.P. (2000). A procedure for an automated measurement of song similarity. *Animal Behaviour, 59*, 1167–1176. (doi:10.1006/anbe.1999.1416)

Veenema, A.H., Bredewold, R., & De Vries, G.J. (2012). Vasopressin regulates social recognition in juvenile and adult rats of both sexes, but in sex- and age-specific ways. *Hormones and Behavior, 61*, 50–56. (doi:10.1016/j.yhbeh.2011.10.002)

Veenema, A.H., Bredewold, R., & De Vries, G.J. (2013). Sex-specific modulation of juvenile social play by vasopressin. *Psychoneuroendocrinology, 38*, 2554–2561. (doi:10.1016/j.psyneuen.2013.06.002)

Voorhuis, T.A.M. & De Kloet, E.R. (1992). Immunoreactive vasotocin in the zebra finch brain (Taeniopygia guttata). *Developmental Brain Research, 69*, 1–10.

Voorhuis, T.A.M., De Kloet, E.R., & De Wied, D. (1991). Effect of a vasotocin analog on singing behavior in the canary. *Hormones and Behavior*, *25*, 549–559.

Warlaumont, A.S., Richards, J.A., Gilkerson, J., & Oller, D.K. (2014). A social feedback loop for speech development and its reduction in autism. *Psycholoical Science*, 0956797614531023. (doi:10.1177/0956797614531023)

West, M.J. & King, A.P. (1988). Female visual displays affect the development of male song in the cowbird. *Nature, 334*, 244–246. (doi:10.1038/334244a0)

Williams H. (2004). Birdsong and singing behavior. *Annals of the New York Academy of Science, 1016*, 1–30. (doi:10.1196/annals.1298.029)

Williams, H., Kilander, K., & Sotanski, M.L. (1993). Untutored song, reproductive success and song learning. *Animal Behaviour, 45*, 695–705. (doi:10.1006/anbe.1993.1084)

Winslow, J.T. & Insel, T.R. (1993). Effects of central vasopressin administration to infant rats. *European Journal of Pharmacology, 233*, 101–107. (doi:10.1016/0014-2999(93)90354-K)

Wood, W.E., Ii, P.J.O., Roseberry, T.K., & Perkel, D.J. (2013). A daily oscillation in the fundamental frequency and amplitude of harmonic syllables of zebra finch song. *PLOS ONE, 8*, e82327. (doi:10.1371/journal.pone.0082327)

Yamamoto, Y., Carter, C.S., & Cushing, B.S. (2006). Neonatal manipulation of

oxytocin affects expression of estrogen receptor alpha. *Neuroscience, 137*, 157–164. (doi:10.1016/j.neuroscience.2005.08.065)

Yamamoto, Y., Cushing, B., Kramer, K., Epperson, P., Hoffman, G., & Carter, C. (2004). Neonatal manipulations of oxytocin alter expression of oxytocin and vasopressin immunoreactive cells in the paraventricular nucleus of the hypothalamus in a gender-specific manner. *Neuroscience, 125*, 947–955. (doi:10.1016/j.neuroscience.2004.02.028)

Zann, R.A. (1996). *The Zebra Finch: A Synthesis of Field and Laboratory Studies*. Oxford University Press, USA.

CHAPTER 6

EVOLVING THE CAPACITY FOR SOCIALLY GUIDED VOCAL LEARNING IN SONGBIRDS

Abstract

Socially guided vocal learning, the ability to use contingent reactions from social partners to guide immature vocalizations to more mature forms, is thought to be a rare ability utilized only by humans, marmosets, and two unrelated songbird species (brown-headed cowbirds and zebra finches). However, this learning strategy has never been investigated in the vast majority of species. To determine which species are most likely to employ socially guided vocal learning, we conducted an analysis of 28 passerines to examine which developmental, reproductive, and ecological traits are predictive of vocal learning systems that incorporate active social guidance. We found three traits to be highly predictive: temporal overlap between the sensory (memorization) and sensorimotor (practice) phases of song learning, using song for mate attraction rather than primarily for territory defense, and being gregarious outside of the breeding season. The species with these traits were distributed throughout the clade. Our model suggests several previously uninvestigated and unexpected species as likely socially guided vocal learners, and offers new insight into the evolution and development of vocal learning.

Introduction

Only seven animal taxa are known to contain vocal learners (Jarvis, 2007). Seemingly rarer still is the capacity for *socially guided vocal learning* (West & King, 1985), a strategy in which the learning organism constructs an imitative outcome via contingent reactions to its immature vocalizations from social partners. Social guidance appears to be vital for vocal development in human infants, whose immature vocalizations are steered towards more mature forms by contingent responses from their caregivers (Goldstein et al., 2003; Gros-Louis et al., 2006; Albert, Schwade & Goldstein, 2017). Currently, socially guided vocal learning has been demonstrated in only three non-human species: marmosets (Chow et al., 2015; Takahashi, Liao & Ghazanfar, 2017), brown-headed cowbirds (West & King, 1988), and zebra finches (Carouso-Peck & Goldstein, 2019a; Carouso-Peck, Menyhart, Devoogd & Goldstein, submitted). What shared traits among these phylogenetically disparate species caused socially guided vocal learning to evolve? Can a species' life history and developmental trajectory predict its vocal learning strategy?

Which other organisms might utilize socially guided vocal learning is largely unknown.. However, just within the last few years, two well-known and well-studied mammal species were serendipitously discovered to be capable of aspects of socially guided vocal learning. Killer whales (*Orcinus orca*) were cross-socialized with bottlenose dolphins during development, and found not only to learn to produce dolphin-like vocalizations, but to alter their use of vocalizations depending on social context (Musser et al., 2014). Similarly, young whales cross-fostered with same-species adults from different vocal dialects were found to adopt the dialect of their

new social partners (Crance et al., 2013). This finding was not the result of a planned experiment, rather it was described by the researchers as an 'adventitious' result of cross-fostering the juveniles for other reasons. A study of vocal turn-taking in common marmosets (*Callithrix jacchus*) observed that vocalizations exhibited marked differences based on social contexts, suggesting they were learned. Subsequent observation found parents provided feedback to their offspring during vocal interactions, guiding learning by vocally responding to context-appropriate vocalizations, and failing to do so when offspring vocalizations were not appropriate (Chow et al., 2015). Young marmosets which receive more vocal feedback from parents contingent on their immature calls also develop more mature calls more quickly (Takahashi, Liao & Ghazanfar, 2017). Like humans, marmosets and killer whales live in cohesive, long-term social units and have strong bonds between mother and offspring, though it is unknown whether these social traits are predictive of or necessary for socially guided vocal learning.

The most commonly studied models of vocal learning are the oscine songbirds, of which approximately 4000 species learn to produce their song. Socially guided vocal learning is well-characterized in only one songbird, the brown-headed cowbird (*Molothrus ater*). Cowbird song is used primarily for attracting females, making female response the most useful and informative social feedback to vocal output during song learning. Female cowbirds selectively respond to the immature song of juvenile males when they produce a song element which is arousing. This response takes the form of a 'wing stroke', a lateral movement of the wing so rapid that it is virtually imperceptible to humans. Juvenile males are motivated to produce song

elements which elicit wing strokes, gradually guiding their song towards a mature form functional for female attraction (West & King, 1988). However, the cowbird's unusual life history as a brood parasite, raised by other species and never exposed to or given the opportunity to learn from their biological parents, previously led many to believe it required unique or innate systems of behavior (Lehrman, 1970; Todd & Miller, 1993). Until recently, socially guided vocal learning was thought to be one such rare evolutionary innovation caused by the cowbird's unusual developmental process. Socially guided vocal learning has rarely been investigated in other oscines, but we recently discovered a similar learning system in the commonly studied zebra finch (*Taeniopygia guttata*), in which non-vocal contingent visual responses from females affect song outcomes in juvenile males (Carouso-Peck & Goldstein, 2019a).

Non-singing female zebra finches learn strong preferences for particular songs (Chen, Clark & Woolley, 2017), and female listeners have long been known to affect song learning in males (e.g. Jones & Slater, 1993; Williams, 2004; Adret, 2004), but the mechanism by which females were influencing males was, until recently, unknown. Although previous researchers have searched for contingent feedback behaviors in this species, none were found when zebra finch behavior was examined in real time (Houx & ten Cate, 1998). This was due to the fact that feedback cues in small birds are often extremely rapid, and often imperceptible to the human eye, with zebra finch wing strokes often lasting less than 0.3 seconds. Our observations of bird behavior in real time are limited by the temporal constraints of human perception, as the human visual system critical flicker fusion rate is about half that of a small bird (Healy et al., 2013). This limitation often led to important but rapid avian behaviors

being overlooked (Carouso-Peck & Goldstein, 2018; Carouso-Peck & Goldstein, 2019b). The black manakin (*Xenopipo atronitens*) was once thought to have a simple courtship display consisting of hopping up and down (Kirwan & Green, 2012). It was only after capturing the display on high-speed video that each hop was found to be an extremely rapid (360 ms) backwards summersault (Lindsay et al., 2015). Similarly, high-speed video revealed that the 'hopping' courtship dance of the cordon bleu finch was in fact a rapid tap dance, alternating the left and right foot (Ota, Gahr & Soma, 2015). The feedback cues required for socially guided vocal learning in many birds are far too fast for a human researcher to perceive unaided. As a result, these cues have rarely been noticed or investigated, and socially guided vocal learning may be a far more ubiquitous learning strategy than previously thought.

Despite their phylogenetic distance, humans, marmosets, orcas, cowbirds, and zebra finches share several traits that may have given rise to socially guided vocal learning as a solution to the problem of developing communicative competence. For example, these species are all socially gregarious, ensuring developmental access to social feedback, and all use their learned vocalizations to facilitate and maintain social bonds. Early in development, each of these species can learn new vocal forms at the same time they are producing immature vocalizations (e.g. Figure 3.1), which may facilitate a role for social feedback in response to immature vocalizations as a means of influencing vocal learning (Carouso-Peck & Goldstein, 2018). In order to investigate whether particular ecological traits may be predictive of the likelihood of a given songbird species to utilize a socially guided vocal learning strategy, we constructed an evolutionary model. Using 28 well-studied passerine species, we conducted a literature search for evidence of social influences on vocal learning, to use

as an outcome measure of susceptibility to social influences on song ontogeny. We investigated a number of ecological, developmental, and reproductive traits to determine which are predictive of the likelihood of a given species to utilize socially guided vocal learning.

Methods

Species Inclusion Criteria

To investigate the prevalence of socially guided vocal learning among passerines, we constructed an evolutionary model to identify which ecological traits may predict the presence of socially guided vocal learning across species. We first performed a broad meta-analysis of the literature describing the ecological traits and song learning trajectories of 114 candidate species of passerine birds using 1,043 papers from the primary literature. Species were only included in analysis if the literature yielded sufficient information about their ecological, developmental, and reproductive traits.

Species chosen for inclusion in the data set were those which passed two criteria. First, species were only included if published experimental data could be found on any of the 13 characteristics on the Social Effects Index (described below). Second, species were only included if sufficient published evidence for scoring could be found on at least five of the six following traits: 1) The primary usage of adult song (territory defense, mate attraction, or both), 2) Whether the sensory and sensorimotor periods of song learning overlap temporally, 3) Whether the species is typically flocking or solitary in the non-breeding season, 4) Whether the species is

migratory/nomadic or sedentary, 5) Whether females of the species typically sing, and 6) Whether the species is an open-ended learner – continuing to learn new song throughout life - or a closed-ended learner - ceasing to learn new song past a certain age (Tables 6.1 and 6.2).

Table 6.1. Summary of literature review of song development, use, evidence for social learning, and Social Effects Index score.
Includes criteria-passing species' lifetime learning timeline (closed- or open-ended learning), presence or absence of temporal overlap in sensory and sensorimotor learning periods, primary use for song (territory defense, mate attraction, or both), and current evidence for or against susceptibility to social influences on vocal learning. Index score was calculated according to the scoring criteria in Figure 6.1.

Species Name	Family	Closed-ended/ Open-ended Learner	Sensory/ Sensori-motor Overlap	Primary Song Use	Social Effects Index Score	Types of Evidence for Social Learning	References
White-crowned sparrow, *Zonotrichia leucophrys*	Emberizidae	Closed-ended	No	Territory defense	Moderate:2	Can learn heterospecific song if deprived of conspecific song, but show preference for conspecific song; learn better from interactive tutor or from passive playback depending on developmental timing of model presentation	*Marler, 1970; Cunningham & Baker, 1983; Brainard & Doupe, 2002; Konishi, 1965; Soha & Marler, 2001a; Soha & Marler, 2001b; Baptista & Petrinovich, 1984; Baptista & Petrinovich, 1986*
Song sparrow, *Melospiza melodia*	Emberizidae	Closed-ended	No	Both	Low: -2	Can learn heterospecific song (white-crowned sparrow); Learn more poorly from interactive tutors than non-interactive tutors; learn well from passive playback	*Marler and Peters, 1987; Nordby, Campbell & Beecher, 2002; Catchpole & Baptista, 1988; Beecher et al., 2007; Nulty et al., 2010*
Swamp sparrow, *Melospiza georgiana*	Emberizidae	Closed-ended	No	Territory defense	Low: -2	Do not learn heterospecific song; learn well from passive playbacks	*Marler & Peters, 1982; Marler & Peters, 1988b; Mooney, 1999; Marler & Peters, 1977*
Dark-eyed junco, *Junco hymalis*	Emberizidae	Closed-ended	No	Both	Moderate: 2	Learn abnormally in isolation; can learn from siblings if deprived of an adult tutor	*Titus, Ketterson & Nolan, 1997; Nolan et al., 2002; Marler, Kreith & Tamura, 1962; Marler, 1966*
Chestnut-sided warbler, *Setophaga pensylvanica*	Parulidae	Closed-ended	Possible overlap for courtship song	Both	High: 4	Can learn heterospecific song; can learn territorial song from tape tutoring; courtship	*Byers & Kroodsma, 1992; Kroodsma et al., 1989; Payne, Payne & Doehlert, 1984; Kroodsma,*

			learning but not territorial song learning			song learning greatly enhanced by live tutoring; will learn songs of peers in absence of tutor	*Meservey & Pickert, 1983*
Red-winged blackbird, *Agelaius phoeniceus*	Icteridae	Open-ended	Yes	Territory defense	Low: -1	Fail to learn in isolation; learn well from passive playback; readily improvise song elements	*Kirn, Clower, Kroodsma & DeVoogd, 1989; Yasukawa, Blank & Patterson, 1980; Marler, Mundinger, Waser & Lutjen, 1972*
Brown-headed cowbird, *Molothus ater*	Icteridae	Closed-ended	Yes	Mate attraction	High: 6	Readily learn from live heterospecifics (canaries); fail to learn in isolation; song learning is known to be socially guided	*O'Loghlen & Rothstein, 2002; Freeberg et al., 2002; Hamilton, King, Sengelaub & West, 1998; King, Freeberg & West, 1996; West & King, 1988*
Northern cardinal, *Cardinalis cardinalis*	Cardinalidae	Closed-ended	Yes	Territory defense	Low: -2	Learn well from passive playback of conspecific song, also invents own song elements	*Lemon & Scott, 1966; Dittus & Lemon, 1969; Yamaguchi, 2001*
Indigo bunting, *Passerina cyanea*	Cardinalidae	Closed-ended	Yes	Both	High: 4	Learn best from interactive tutors; do not learn well from passive playback; fail to learn in isolation	*Payne, 1981; Rice & Thompson, 1968; Thompson, 1970; Thompson, 1972; Beckett & Ritchison, 2010*
Atlantic canary, *Serinus canaria*	Fringillidae	Open-ended	Yes	Mate attraction	High: 4	Fail to learn in isolation; learn best from live interactive tutors; fail to learn from passive playback; learns song from peers in absence of tutor; may improvise song if untutored	*Nottebohm, Nottebohm & Crane, 1986; Poulsen, 1959; Nottebohm & Nottebohm, 1978; Waser & Marler, 1977; Leitner & Catchpole, 2007; Belzner, Voigt, Catchpole & Leitner, 2009*
Common chaffinch, *Fringilla coelebs*	Fringillidae	Closed-ended	Yes	Both	High: 4	Readily learn from live heterospecifics (canaries); fail to learn in isolation; learn well from interactive tutors but also learn calls and songs from passive playback	*Nottebohm, 1971; Thielcke & Krome, 1991; Slater & Ince, 1982; Thorpe, 1958; Thorpe, 1961; Riebel & Slater, 1998*
Eurasian bullfinch, *Pyrrhula pyrrhula*	Fringillidae	Closed-ended	Yes	Mate attraction	High: 4	Readily learn from live heterospecifics (i.e. canaries, preferred over non-interactive conspecific); learn best from interactive tutors	*Thorpe, 1958; Nicolai, 1959; Newton, 2000*
Zebra finch, *Taeniopygia guttata*	Estrildidae	Closed-ended	Yes	Mate attraction	High: 7	Readily learn from live heterospecifics (e.g. Bengalese finches); fail to learn well in	*Immelmann, 1969; Brainard & Doupe, 2002; Zann, 1996; Sossinka & Böhner, 1980; Williams,*

						isolation; can potentially learn from non-social and social contingency; learn best from live interactive tutors; do not learn well from passive playback	*Kilander & Sotanski, 1993; Price, 1979; Adret, 1993; Carouso-Peck & Goldstein, 2019; Williams, 2004; Eales, 1989*
Bengalese finch, *Lonchura striata domestica*	Estrildidae	Closed-ended	Yes	Mate attraction	High: 5	Learn well from live interactive tutors; readily learn from live heterospecifics; can learn from non-social contingent cues	*Immelmann, 1969; Sober & Brainard, 2009*
Marsh wren, *Cistothorus palustris*	Troglodytidae	Closed-ended	No	Both	Low: 0	Learn well from interactive tutors, but also from passive playback; copy neighbor song in wild	*Kroodsma, 1986; Kroodsma & Verner, 1987; Verner, 1975*
Sedge wren, *Cistothorus platensis*	Troglodytidae	Closed-ended	Yes	Territory defense	Low: 1	Fail to learn from passive playback, instead improvising song; learn from siblings/peers	*Kroodsma, Liu, Goodwin & Bedell, 1999; Kroodsma et al., 1999; Kroodsma & Verner, 1978*
Common treecreeper, *Certhia familiaris*	Certhiidae	(Debated)	Yes	Both	High: 5	Can learn from heterospecifics; fail to learn in isolation; do not learn from passive playback, only live tutors; can learn from siblings/peers	*Tietze, Martens, Sun & Päckert, 2008; Thielcke, 1986; Thielcke, 1977; MackKay, 2001; Thielcke, 1984; Thielcke & Wustenberg, 1985*
Black-capped chickadee, *Poecile atricapillus*	Paridae	Closed-ended	Yes	Both	High: 5	Can learn heterospecific song; fail to learn from passive playback; learn well from live tutors (social interaction seems vital for learning)	*Kroodsma, Albano, Houlihan & Wells, 1995; Grava et al., 2013; Shackleton & Ratcliffe, 1933; Baker, Baker & Gammon, 2002*
Sedge warbler, *Arcocephalus schoenobaenus*	Acrocephalidae	Open-ended	Yes	Both	Low: -3	Learn to sing in isolation (probably improvising)	*Nicholson, Buchanan, Marshall & Catchpole, 2007; Leitner et al., 2002*
Grey catbird, *Dumetella carolinensis*	Mimidae	Open-ended	Yes	Both	Low: -1	Can learn from heterospecifics (mimicry); do not fail to learn in isolation	*Kroodsma, Houlihan, Fallon & Wells, 1997; Dolby et al., 2005*
European starling, *Sturnus vulgaris*	Sturnidae	Open-ended	Yes	Mate attraction	High: 6	Readily learn from live heterospecifics; fail to learn in isolation; learn best from interactive tutors; fail to learn well from passive playback	*Brenowitz & Beecher, 2005; Adret-Hausberger, Güttinger & Merkel, 1990; Chaiken, Böhner & Marler, 1994; Chaiken, Gentner & Hulse, 1997; Mountjoy & Lemon, 1995; West, Stoud & King, 1983;*

							Poirier et al., 2004; Chaiken, Böhner & Marler, 1993
Northern mockingbird, *Mimus polyglottos*	Sturnidae	Open-ended	Yes	Both	Low: 1	Readily learn from heterospecifics (mimic)	*Brenowitz & Beecher, 2005; Derrickson, 1987; Howard, 1974*
American robin, *Turdus migratorius*	Turdidae	Closed-ended	No	Both	Moderate: 2	Fail to learn normally in isolation but do improvise elements; do not learn well from passive playback	*Johnson, 2006; Eisrer, 1976*
Nightingale, *Luscinia megarhynchos*	Muscicapidae	Open-ended	Yes	Both	High: 4	Readily learn from heterospecifics (mimic); learn best from interactive tutors (even interactive humans); do not learn well from passive playback	*Keifer et al., 2009; Kiefer, Sommer, Scharff & Kipper, 2010; Hultsch & Todt, 1989; Bartsch, Weiss & Kipper, 2015; Amrhein, Kunc & Naguib, 2004; Todt, Hultsch & Heike, 1979*
Pied flycatcher, *Ficedula hypoleuca*	Muscicapidae	Open-ended	No	Both	Moderate: 2	Can learn heterospecific song if cross-fostered (by great tits or blue tits)	*Espmark & Lampe, 1993; Skjerven, 2011; Sirkiä & Laaksonen, 2009; Eriksen, Lampe & Slagsvold, 2009*
Albert's lyrebird, *Menura alberti*	Menuridae	Open-ended	Yes	Both	Low: 1	Learn well from heterospecifics (mimic)	*Robinson & Curtis, 1996*
Three-wattled bellbird, *Procnias triarunculatus*	Cotingidae	Open-ended	Yes	Both	Moderate: 2	Can learn from live heterospecifics and can learn multiple dialects	*Kroodsma et al., 2013; Snow, 1977*
New world flycatchers (Alder and Willow flycatcher), *Empidonax alnorum* and *Empidonax traillii*	Tyrannidae	No learning	No sensitive periods	Territory defense	Low: -3	Do not learn from heterospecifics; learn normal song in isolation; do not learn from tutors (song appears to be innate)	*Kroodsma, 1984*

Table 6.2. Summary of literature review of three ecological traits included in model construction.
Includes the criteria-passing species' migratory tendencies (resident, migratory, or semi-migratory/nomadic), capacity for song in females, and average non-breeding flock size. Only flock size was predictive of Social Effects Index score and included in the final model. Data were omitted for species for which data was not available or available information varied widely between peer-reviewed sources.

Species	Migratory habits	Females sing?	Flock size	References
White-crowned sparrow	Primarily migratory (4 of 5 subspecies)	Yes	3-10, larger winter flocks	*Nelson & Marler, 1994; Baptista, Trail, DeWolfe & Morton, 1993; Slowtow & Rothstein, 1995*
Song sparrow	Resident	Rarely	Territorial year-round	*Wingfield, 1994; Arcese, Stoddard & Hiebert, 1988; Goodson, Wilson & Schrock, 2012*
Swamp sparrow	Migratory	No	Small (3-11), mixed-species flocks	*Boyle, 2011; Greenberg, Olsen & Etterson, 2010; Marler & Peters, 1977*
Dark-eyed junco	Migratory	Rarely, urban populations only	Small (15-25)	*Bent, 1978; Rogers, Theimer, Nolan & Ketterson, 1989; Deviche & Gulledge, 2000; Reichard, 2017*
Chestnut-sided warbler	Migratory	Yes	Large, mixed-species	*Byers & King, 2000; Ficken & Ficken, 1965; Greenberg, 1984*
Red-winged blackbird	Semi-migratory	Yes	Very large (can flock in millions)	*Dolbeer, 1978; Meanley, 1965; Beletsky, 1983*
Brown-headed cowbird	Migratory	No	Large (mixed species, sometimes >100,000)	*185-188 Ortega, 1998; Johnson et al., 1980; Smith, King & West, 2000; King & West, 1983*
Northern cardinal	Resident	Yes	Small (12-24)	*Bent, 1968; Ritchison, 1986; Wolfenbarger, 1998; Halkin & Linville, 1999*
Indigo bunting	Migratory	No	Large	*Bent, 1968; Payne, 1991; Payne, 1981*
Atlantic canary	(Domesticated), wild canaries do not migrate	No	Highly social, domesticated	*Clarke, Orgill & Dudley, 2006; Brown & Bottjer, 1993*
Common chaffinch	Semi-migratory	No	Large (max 1500), mixed	*Bertold, 2001; Nottebohm, 1966; Swann, 1988*
Eurasian bullfinch	Semi-migratory	Yes	Small, loose flocks	*Wilkinson, 1990; Nottebohm, 1972; Summers, 1979; Newton, 1967*
Zebra finch	Semi-migratory (nomadic)	No	50 (breeding) to 100 (non-breeding)	*Zann, 1996*
Bengalese finch	(Domesticated)	No	Highly social (domesticated)	*Wooley & Rubel, 1997*
Marsh wren	Resident	No	Solitary	*Verner, 1965; Welter, 1935; Verner, 1975*
Sedge wren	Seminomadic	No	Solitary	*Bibby & Green, 1981; Herkert, Kroodsma & Gibbs, 2001*
Common treecreeper	Resident	No	Small (9 average, 35 max)	*Bertold, 2001; Arevalo & Gosler, 1994; Jäntti, Hakkarainen, Kuitunen &*

				Suhonen, 2007
Black-capped chickadee	Resident	Yes	Small (6-8 bird mixed flocks in winter, pairs in summer)	*Gayk & Lindsay, 2012; Hahn, Krysler & Sturdy, 2013; Harrap, 1996*
Sedge warbler	Migratory	No	Solitary	*Bensch, 1999; Kennerley & Pearson, 2010*
Gray catbird	Migratory	Yes (quietly)	Solitary	*Cimprich & Moore, 1995; Harcus, 1973*
European starling	Semi-migratory	Yes	Large	*Kessel, 1953; Pavlova, Pinxten & Eens, 2005*
Northern mockingbird	Semi-migratory	Yes	Solitary	*Laskey, 1936; Derrickson & Breitwisch, 1992*
American robin	Migratory	Yes	Solitary	*Wauer, 1999; Aldrich & James, 1991*
Nightingale	Migratory	Yes	Solitary	*Emmenegger, Hahn & Bauer, 2014; Hultsch & Todt, 1989*
Pied flycatcher	Migratory	-	Solitary	*Beaman & Madge, 2010; Both, Bijlsma & Visser, 2005*
Albert's lyrebird	Resident	No	Solitary	*Curtis, 1972; Higgins, Peter & Steele, 2001*
Three-wattled bellbird	Migratory	No	Solitary	*Snow, 1982; Powell & Bjork, 2003; Kroodsma et al., 2013*
New-world flycatchers	Migratory	Yes	Solitary	*Jahn et al., 2013; Mathewson, Morrison, Loffland & Brussard, 2012; Peabody, 1929; Sedgwick, 2004*

Constructing the Social Effects Index

To assess the likelihood of songbird species using social feedback in their song development, we then performed a second, independent literature search for experimental evidence of susceptibility to social influences on learning within our sample of 114 species. We found that social effects on learning have only been investigated in a small subset of song-producing passerines. From these data we constructed a Social Effects Index (hereafter 'Index') as our dependent variable. The Index integrated various types of evidence for or against social effects on learning, to produce a single score reflecting the likelihood of social influences on learning for each species (for all characteristics used, see Figure 6.1 and Table 6.1). None of the traits used to make the regression model overlapped with the song and learning

characteristics used for construction of the Social Effects Index. Characteristics used for the Index were those any behavioral, developmental, or learning tendencies of a species which suggested the presence or absence of social influences on vocal learning, such as ability or inability to learn from passive playback, in isolation, via eavesdropping on adult conspecifics, or from interactive tutors. This resulted in a data set supporting both positive and negative effects of social learning across a subset of 28 relatively well-studied species (Figure 6.2).

Each species was assigned a 'yes', 'no', or 'unknown' value on each of the 13 learning characteristics included in the Index. Each characteristic was assigned a positive value (if it suggested evidence for utilization of social feedback) or a negative value (if it suggested evidence against utilization of social feedback). Most characteristics were given +1 or -1 values on the Index, but characteristics which demonstrated very strong evidence for or against social learning were given higher +2 or -2 values (Figure 6.1). Weights for each trait in the Index (Figure 6.1) were determined based on the degree to which each trait suggested the presence or absence of social influences on vocal learning. For example, the finding that a given species learns song better from an interactive tutor than from passive playback of song is strong evidence that some form of interactive social feedback facilitates song learning, so this characteristic is assigned a +2 value on the Index. In contrast, the ability of a species to learn heterospecific song when presented with a live heterospecific tutor suggests a strong influence of social factors on learning, but the inability of a species to learn heterospecific song does not as strongly indicate that social factors are not important to learning conspecific song, and these characteristics were accordingly

assigned values of +2 and -1, respectively. The Index value for each species was calculated by adding together the values associated with each characteristic on the Index found to be identified in the species.

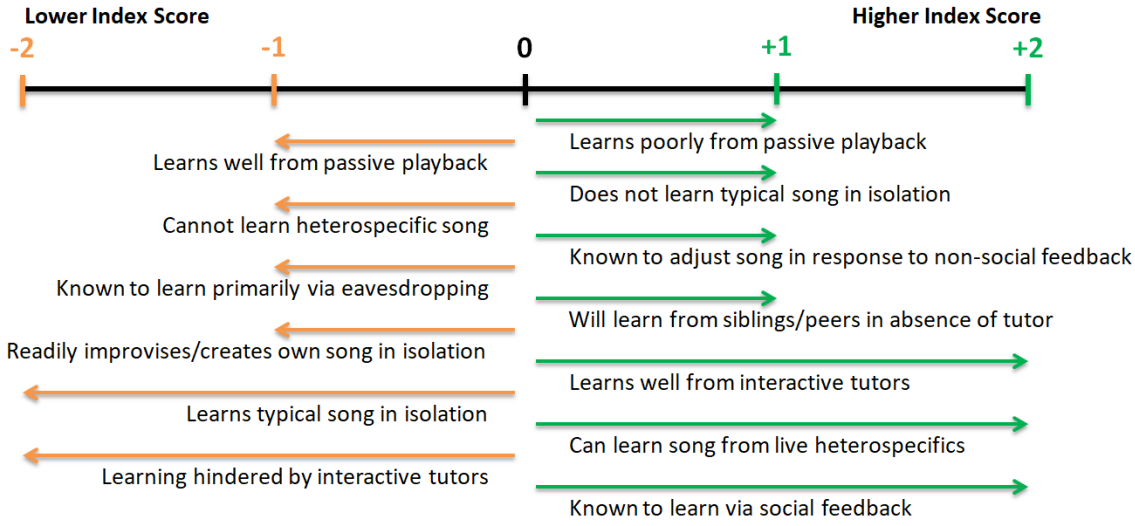

Figure 6.1. Traits and their respective weights contributing to the Social Effects Index measure of susceptibility to social influences on learning.
In order to construct the Social Effects Index measure of susceptibility to social influences on learning, each characteristic was assigned a positive (green arrows) or negative (orange arrows) value, respectively, if it suggested evidence for or against socially guided vocal learning. Most characteristics were given +1 or -1 values, but traits demonstrating very strong evidence for or against socially guided vocal learning were given +2 or -2 values (see *Methods*). The final Index score for each species was calculated from the sum total of their trait values.

Regression and Phylogenetic Trees

We used the package 'rpart' in R software (Therneau & Atkinson, 2009) to build our regression model by splitting the dataset into homogenous subsets of Index score using a single predictor variable at each node. The initial result was a large tree incorporating all six ecological trait variables, which could be over-fitted. We therefore pruned the tree to an optimal size using results from 8 cross-validations

(Breiman, Friedman, Stone, & Olshen, 1984) in order to find an optimal trade-off between complexity of the model and accuracy of predictions. For our relatively small sample, we did not choose the minimum cross-validated error, but instead one which was within one standard deviation of the prediction error. This resulted in the best possible model based on cross-validation, a simple tree with 2 splits (Figures 6.3A and 6.3B). The phylogenetic tree (Figure 6.2) was based on data reconstructed for maximum clade credibility based on molecular data (Sibley & Ahlquist, 1990; Barker, Barrowclough & Groth, 2002 62) with updated data estimated by Bayesian MCMC analyses of concatenated mitochondrial and nuclear intron sequences in New World species (Barker et al., 2015) and Mimidae (Lovette et al., 2012). We then verified our tree using recent avian phylogenetic data (Jetz et al. 2012, Jetz et al., 2014) on birdtree.org, and constructed it using Interactive Tree of Life (ITOL; Letunik & Bork, 2016).

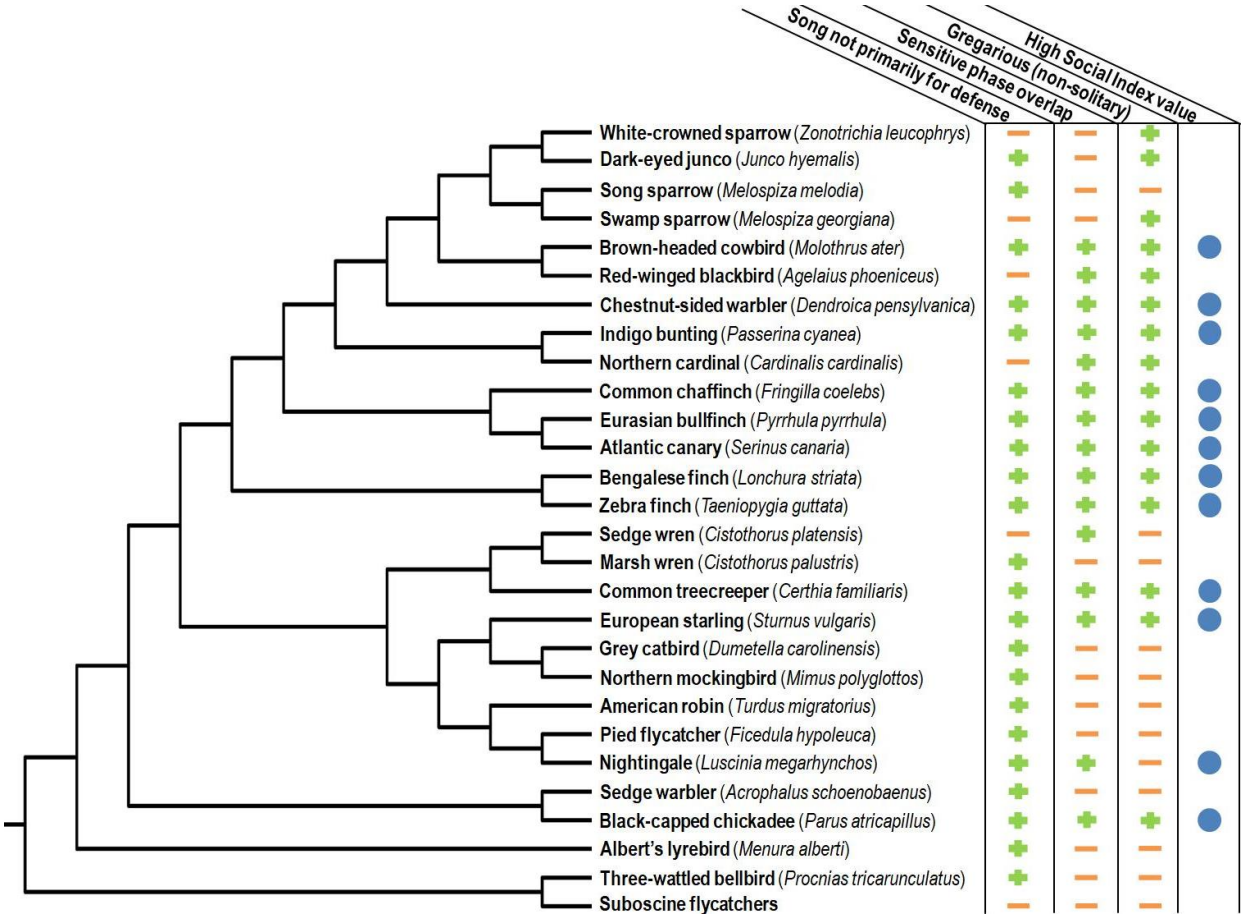

Figure 6.2. Cladistic tree of passerine species included in the final evolutionary model of ecological traits and social learning susceptibility.
28 passerine species with ecological trait presence (green +) or absence (orange -) for the three primary predictive traits included in the final model. From left to right, these are: primary use of song not being territory defense (song is used to at least an equal degree for mate attraction); existing temporal overlap in the sensory memorization and sensorimotor practice phases of song learning; gregariousness, as defined by being non-solitary in the non-breeding season. Blue circles in the final column (far right) indicate high (≥4) scored on the Social Effects Index. This tree is intended to demonstrate relatedness between modern species, but branch lengths are not indicative of actual temporal points of speciation as we were not attempting to determine maximum-likelihood ancestral state reconstruction (see *Methods*).

Results

Species with high Index scores (higher than the median split value of ≥ 4) typically had several traits in common, including temporal overlap in sensory and sensorimotor phases of song learning, learning duration, primary function of song, migratory tendencies, gregariousness/flocking tendencies, and absence of female song. We conducted regressions to examine the joint and unique contributions of these traits to numerical Index score. Each species was placed in the category of social learning likelihood rated 'high' (Index score ≥ 4), 'moderate' (Index score of 2 to 3) or 'low' (Index score < 2).

We first ran chi-squares on all identified traits in isolation, in order to ascertain whether a relation existed between a given trait value and score on the Index. Of the traits examined, only three were related to the dependent variable of Index score: temporal overlap in sensory and sensorimotor learning periods, primary use of song, and tendency to form flocks (gregariousness). Species with some degree of overlap between sensory and sensorimotor learning periods were found to be significantly more likely to be scored 'high' on the Index (see *Methods*), while species without evidence of overlap were significantly more likely to be scored 'low' or 'moderate' (χ^2 (2) = 11.607, p = .003). Species which use song primarily for territory defense were significantly more likely to be 'low' on the Index (these species were never in the 'high' category), those which use song primarily for mate attraction were significantly more likely to be rated 'high' (these species were never found in the 'low' or 'moderate' categories), and those which use song for both purposes were equally likely to fall into the 'high' and 'low' categories (χ^2 (4) = 14.117, p = .007).

Species with the tendency to flock during the non-breeding season were found to be significantly more likely to be 'high' on the Index, compared to primarily solitary species which were significantly more likely to be 'low' on the Index (χ^2 (2) = 10.448, p = .005).

We then ran Pearson's correlations between the presence of each trait and total Index score for each species, and found the same three traits to be significantly correlated with the outcome measure: primary song use (r (26) = .522, p = .004), gregariousness/flocking tendencies (r (26) = .513, p = .005), and sensory/sensorimotor overlap (r (26) = .421, p = .026). When nonparametric tests were run using Index score as a continuous variable, presence of sensory/sensorimotor overlap was significantly related to Social Effects Index score (Mann-Whitney U = 37.5, p = .028) as were primary song use (U = 19, p = .007) and flock size (U = 35, p = .004). Only significant predictors were included in the final model (Figure 6.3A).

We then modeled the manner in which these traits interact in the evolution of species with high Index scores and conducted regressions to examine the joint and unique contributions of these ecological traits to numerical Index score. Sensory/sensorimotor overlap was in isolation a significant predictor of Index score (R^2 = .177, p = .026), but adding the traits of primary song use and gregariousness resulted in a stronger model (R^2 = .616, p < .0001) (Figures 6.3A and 6.3B). Our model is supported by the finding that cowbirds and zebra finches, the only two species in which socially guided vocal learning has yet been demonstrated, each exhibit all three of the identified predictive traits. Finally, we modeled the manner in which these traits interact in the evolution of species with high Index scores. A

regression tree revealed that primary use of song is the most predictive factor, while sensory/sensorimotor overlap is necessary but not sufficient for incorporation of social factors into vocal development (Figure 6.3).

The regression tree of our evolutionary model (Figure 6.3B) begins with 100% of our sample (n = 28), with a mean Index score of 2. Species which use their song primarily for territory defense consistently had low evidence of social learning (21% of sample, Node 1) compared to those which do not (79% of sample, Node 2). Within species which do not use song primarily for territory defense, gregariousness predicts Index score. Species which also do not flock or are solitary in the non-breeding season had lower Index scores (36% of sample, Node 3) than those which are flocking and gregarious year-round (43% of sample, Node 4).

The model also revealed several traits that did not predict the Index score. Whether a species was migratory/nomadic or sedentary was not significantly predictive of Index value, (χ^2 (2) = 2.461, p = .292) nor was presence or absence of song in females, (χ^2 (2) = .054, p = .973) or age-limited versus open-ended song learning, (χ^2 (2) = 1.095, p = .579).

A

Model	R^2	R^2 Change	Beta	Sig.
Phase Overlap	.177	.177	.421	.026*
Phase Overlap + Song Use	.354	.177	.312 .435	.072 .015*
Phase Overlap + Song Use + Gregariousness	.616	.262	.155 .528 .536	.265 .001*** .000***

B

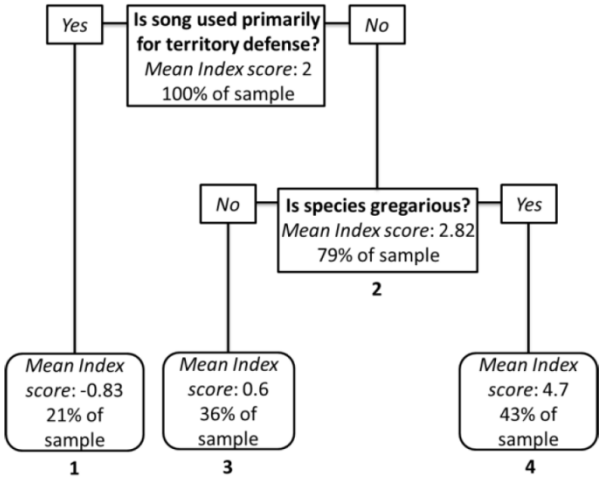

Figure 6.3. Predictive model of Social Effects Index based on ecological traits.
(A) Three regression models of traits predicting Social Effects Index score and associated change in R^2 values. The combined model incorporating overlap of the sensory and sensorimotor developmental learning phases, the primary use of song (used for territory defense or not), and gregariousness (measured by flock size in the non-breeding season) best predicted Index scores across species, * $p < .05$, *** $p < .001$. (B) Regression tree of model predicting Social Effects Index from three predictive ecological traits. Species which use song primarily for territory defense have low Social Effects Index scores (Node 1, mean Index score = -0.83) compared to those which do not (Node 2, mean Index score = 2.82). Within species which do not use song primarily for territory defense, gregariousness predicts Index score, with non-gregarious species (Node 3, mean Index score = 0.6) having lower Index scores than more highly gregarious species (Node 4, mean Index score = 4.7).

Discussion

We found three traits – gregariousness, using song for functions other than territory defense, and having developmental overlap between the memorization and practice phases of song acquisition – to be strongly predictive of a given species' susceptibility to social influences on vocal learning. Our model is supported by the recent finding of socially guided vocal learning in a species, the zebra finch, that exhibits all three of these traits.

The possibility for a socially guided vocal learning strategy appears to be highly dependent on whether young birds are developing an expectation of what their song should sound like at the same time they are practicing motor production of their immature songs. None of the sampled species with no sensory/sensorimotor overlap had a high likelihood of requiring social feedback for song learning. These two phases of learning vary widely in their degree of developmental overlap between species (Brainard & Doupe, 2002). In most seasonally breeding species, including many sparrows, these phases are temporally distinct, with memorized representations of adult song forming during the sensory phase shortly after hatching in the spring, and sensorimotor practice only commencing in the autumn (Marler, 1970). Sparrows therefore learn to produce whatever was memorized earlier in life, and any social feedback received based on their immature song is unable to alter this template-like representation. An alternative developmental trajectory for song learning exists in numerous species, including both zebra finches and brown-headed cowbirds, in which the timing of the sensory and sensorimotor periods overlap substantially (see Table 6.1 and Figure 3.1). This creates the opportunity for social feedback to immature song to affect the young bird's mental representation of the song it should ultimately produce, though to our knowledge the link between this sensitive phase overlap and social influences on vocal learning has never been comparatively investigated.

Similarly, none of the species which used their song primarily for territory defense had a high degree of evidence for social learning. Given that highly territorial species tend to learn song via eavesdropping on neighbors, and risk aggressive encounters if they venture too near to their tutors, the necessity for social learning

would be maladaptive in these species (e.g. Otter et al., 1999). The forms of socially guided vocal learning thus far discovered have primarily involved adult females selectively responding to the immature song of juvenile males (West and King, 1988; Carouso-Peck, Menyhart, Devoogd & Goldstein, submitted). This form of learning necessitates song which is ultimately used for mate attraction, as it must generate an arousal response from females to drive learning. Brown-headed cowbirds are non-territorial and highly gregarious, using their song primarily for attracting a mate. In such a system, eavesdropping on other males could lead to reproductively disastrous learning outcomes, as a young male has no means of assessing which overheard songs are most functional. Given that females, not males, will be the ultimate judges of the learned song, a more adaptive strategy evolved to allow juvenile males to alter their immature song in response to female feedback. The same appears to be the case in zebra finches, a non-territorial, gregarious species which primarily uses song for female attraction. As in the cowbird, song appears to serve little purpose for intrasexual communication in zebra finches, making intersexual feedback a more reliable guide towards reproductively functional song. 100% of the sampled species with high ratings of evidence of social factors on learning had both some degree of overlap in their sensory and sensorimotor periods and used their song primarily for mate attraction or both mate attraction and territory defense together, but not territory defense primarily.

Use of song for territory defense seemed to be related to the ability to learn passively. All eight of the species in our sample which demonstrated the ability to learn readily from passive playback of tape-recorded conspecific song also use song

for territory defense (Table 6.2). This passive learning capacity appears to rely on the species' natural mode of learning, with species which typically learn via eavesdropping on territorial neighbors able to learn from passive exposure, while those which learn via direct interactions with conspecific tutors require interactive feedback. Canaries (*Serinus canarius*), European starlings (*Sturnus vulgaris*), and indigo buntings (*Passerina cyanea*) all learn via interaction with conspecifics, and fail to learn in the absence of an interactive model (Waser & Marler, 1977; Chaiken, Böhner & Marler, 1993; Rice & Thompson, 1968). In contrast, similarly well-studied species such as the song sparrow (*Melospiza melodia*) have been shown not to benefit from social interaction during song learning, and even to be hindered by it in some cases. Young song sparrows learned half as many songs from an adult tutor in the same cage as from a tutor they could overhear in a distant cage (Beecher et al., 2007). This is likely due to eavesdropping functioning as the primary natural mode of vocal learning in most sparrows. Since sparrow song is used primarily for territory defense and aggressive interactions, young sparrows identify and imitate the most aggressive local adult male by passively listening to his song. Being near enough to the tutor to enable social interaction would require entering his territory, eliciting potentially lethal aggression, making one-on-one interactive tutoring untenable as a strategy for this species in the wild.

However, social factors usually have some degree of impact on vocal learning in a songbird, even among the species with low scores on the Index. According to the action-based learning model, juvenile sparrows may adjust their repertoire depending on vocal interactions and retain elements that elicit reactions from the receivers, their

father or conspecific neighbors (Nelson & Marler, 1994). Most species are far less discriminating than sparrows in their choice of tutors and will even readily learn from heterospecifics under certain conditions. This capacity varies even between varieties of sparrows, as swamp sparrows cannot copy tape recordings of heterospecific song elements, but the closely related song sparrows will do so (Marler & Peters, 1988a). For zebra finches and cowbirds, the main criterion for tutor selection is live interaction. Zebra finches learn accurate copies of Bengalese finch song (Immelmann, 1969), and cowbirds will learn canary song (King et al., 1996), provided that they are housed with these species during their sensitive learning period. Another group, the mimics - including marsh warblers, lyrebirds, brown thrashers, and northern mockingbirds – are capable of copying most sounds they hear, even those made by non-avian species or anthropogenic noise. Even in these species, learning appears to be modulated by social factors. In the case of the nightingale, live tutors presented early in life are vital for normal song acquisition, and even a human with a loudspeaker is sufficient provided that they are visible to the bird (Todt et al., 1979). For some species the most important factor in determining which song to learn is some inherent species-specific quality of the song. In the case of the white-crowned sparrow, heterospecific song can be learned if it is preceded by a white-crowned-sparrow-like whistle (Soha & Marler, 2000). Territorial species which rely on eavesdropping are more likely to have these species-specific gating mechanisms on learning, potentially to avoid accidental copying of the wrong species (Thielcke, 1986; Beecher et al., 2007). For species which learn via direct interaction with conspecifics, this danger is considerably reduced, and vocal flexibility is enhanced by the absence

of such a gating mechanism. Therefore, the primary use of song in a given species appears to impact social learning opportunities and resulting learning strategy.

Species with the three predictive traits are distributed in their phylogenetic relatedness, indicating an important role of convergent evolution in the emergence of socially guided vocal learning (Figure 6.2). It has been speculated that vocal learning strategy in primates might be affected by life history traits, such as prosociality and cooperative breeding (Snowdon, Elowson & Roush, 1997; Ghazanfar, Liao & Takahashi, 2019). New World primates, especially callitrichids, are more likely to be cooperative breeders than Old World primates, making it difficult to separate trait from phylogeny in this clade. Expanding investigations into avian socially guided vocal learning beyond closely related species, using clues from shared aspects of life histories, could lead to the discovery of new learning strategies in unexpected clades. The brown-headed cowbird, a member of the Icteridae family, also exhibits the three predictive traits, and was the first passerine in which socially guided vocal learning was identified (West & King, 1988). A strictly phylogenetic approach to identifying other social learners would point us towards closely related Icterids such as the red-winged blackbird. However, this species, unlike the cowbird, is an open-ended learner, highly territorial, and readily learns from passive playback of tape recorded songs, with little evidence that it requires any social feedback to learn song. The apparent actively guided social learners are instead scattered across the phylogenetic tree, indicating that shared life history, rather than phylogenetic relatedness, is predictive of learning strategy.

Conclusion

We emphasize that there is no universal vocal learning strategy across species (Beecher & Brenowitz, 2005). Social ecology traits such as cooperative breeding have previously been effectively used to predict vocal complexity across species (Leighton, 2017), but never to predict vocal learning strategy. Our model demonstrates that three traits – gregariousness, use of song for mate attraction rather than territory defense, and overlap in the sensory and sensorimotor stages of song development – are predictive of evidence of social influences on song learning. As we have shown, developmental data on socially guided vocal learning can inform evolutionary models of species ecological traits, and vice versa. Such data are sparse, however, as few researchers have looked for influences of structured social interaction on vocal learning (West & King, 1985). In the continuing search for those species which, like humans, utilize socially guided vocal learning, we must look beyond the traditionally studied temperate zone seasonally breeding species. We must also search beyond those species closely related to cowbirds and zebra finches, instead focusing on ecological traits predictive of social learning which may have independently evolved. Our evolutionary model predicts that certain passerine species (chestnut-sided warblers, indigo buntings, Atlantic canaries, common chaffinch, Eurasian bullfinch, Bengalese finch, common tree creepers, black-capped chickadees, European starlings, nightingales) are most likely to show evidence of socially guided vocal learning. We call for further investigation into these oscines, as the role of socially guided vocal learning in their vocal ontogeny is currently unknown. Many of these species potentially offer useful parallels to aspects of human speech learning. For example,

Bengalese finch (Honda & Okanoya, 1999), black-capped chickadees (Hailman & Ficken, 1986), and European starlings (Gentner, Fenn, Margoliash & Nusbaum, 2006) learn songs characterized by a syntax containing probabilistic elements. Also, unlike sparrows and zebra finches, several of these species are open-ended learners like humans (Figure 3.1). In addition to presenting new model systems for the evolution and development of human language, investigating the prevalence of socially guided vocal learning across vocal learners will offer insights into the evolution of such a socially-embedded learning strategy.

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REFERENCES

Adret, P. (1993). Operant conditioning, song learning and imprinting to taped song in the zebra finch. *Animal Behaviour, 46*, 149–159.

Adret, P. (2004). Vocal imitation in blindfolded zebra finches (*Taeniopygia guttata*) is facilitated in the presence of non-singing females. *Journal of Ethology, 22*, 29-35.

Adret-Hausberger, M., Güttinger, H-R., & Merkel, F.W. (1990). Individual life history and song repertoire changes in a colony of starlings (*Sturnus vulgaris*). *Ethology, 84*, 265–280.

Albert, R., Schwade, J.A., & Goldstein, M.H. (2017). The social functions of babbling: Acoustic and contextual characteristics that facilitate maternal responsiveness. *Developmental Science, 18*, 1-11.e12641.

Aldrich, J.W. & James, F.C. (1991). Ecogeographic variation in the American robin (*Turdus migratorius*). *Auk, 108*, 230-249.

Amrhein, V., Kunc, H.P., & Naguib. M. (2004). Non–territorial nightingales prospect territories during the dawn chorus. *Proceedings of the Royal Society B, 271*, S167–S169.

Arcese, P., Stoddard, P.K., & Hiebert, S.M. (1988). The form and function of song in female song sparrows. *Condor, 90*, 44-50.

Arevalo, J.E. & Gosler, A.G. (1994). The behaviour of treecreepers Certhia familiaris in mixed-species flocks in winter. *Bird Study, 41*, 1-6.

Baker, M.C., Baker, M.S.A., & Gammon, D.E. (2002). Vocal ontogeny of nestling and fledgling black-capped chickadees *Poecile atricapilla* in natural populations. *Bioacoustics, 13*, 265-295.

Baptista, L.F. & Petrinovich, L. (1984). Social interaction, sensitive phases and the song template hypothesis in the white-crowned sparrow. *Animal Behaviour, 32*, 172–181.

Baptista, L.F. & Petrinovich, L. (1986). Song development in the white-crowned sparrow: social factors and sex differences. *Animal Behaviour, 34*, 1359–1371.

Baptista, L.F., Trail, P.W., DeWolfe, B.B., & Morton, M.L. (1993). Singing and its functions in female white-crowned sparrows. *Animal Behaviour, 46*, 511-524.

Barker, F.K., Barrowclough, G.F., & Groth, J.G. (2002). A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. *Proceedings of the Royal R Society B, 269*, 295-308.

Barker, F.K., Burns, K.J., Klicka, J., Lanyon, S.M., & Lovette, I.J. (2015). New insights into New World biogeography: An integrated view from the phylogeny of blackbirds, cardinals, sparrows, tanagers, warblers, and allies. *Auk, 132*, 333-348.

Bartsch, C., Weiss, M., & Kipper, S. (2015). Multiple song features are related to paternal effort in common nightingales. *BMC Evolutionary Biology, 15*, 115.

Beaman, M. & Madge, S. (2010). *The Handbook of Bird Identification for Europe and the Western Palearctic* (A&C Black Publishers, London).

Beckett, M.D. & Ritchison, G. (2010). Effects of breeding stage and behavioral context on singing behavior of male indigo buntings. *Wilson Journal of Ornithology, 122,* 655–665.

Beecher, M.D., Burt, J.M., O'Loghlen, A.L., Templeton, C.N., & Campbell, S.E. (2007). Bird song learning in an eavesdropping context. *Animal Behaviour, 73,* 929–935.

Beletsky, L.D. (1983). Aggressive and pair-bond maintenance songs of female red-winged blackbirds (*Agelaius pboeniceus*). *Zeitschrift fur Tierpsychologie, 62,* 47-54.

Belzner, S., Voigt, C., Catchpole, C.K., & Leitner, S. (2009). Song learning in domesticated canaries in a restricted acoustic environment. *Proceedings of the Royal Society B, 276,* 2881–2886.

Bensch, S. (1999). Autumn migration speed of juvenile reed and sedge warblers in relation to date and fat loads. *Condor, 101*, 153-156.

Bent, A. (1968). *Life Histories of North American Cardinals, Grosbeaks, Buntings, Towhees, Finches, Sparrows, and Allies: Part II* (New York: Dover Publications).

Bertold, P. (2001). *Bird Migration: A General Survey* (Oxford University Press, Oxford).

Bibby, C.J. & Green, R.E. (1981). Autumn migration strategies of reed and sedge warblers. *Ornis Scandinavica, 12,* 1-12.

Both, C., Bijlsma, R.G., & Visser, M.E. (2005). Climatic effects on timing of spring migration and breeding in a long-distance migrant, the pied flycatcher *Ficedula hypoleuca*. *Journal of Avian Biology, 36*, 368-373.

Boyle, W.J. (2011). *The Birds of New Jersey: Status and Distribution* (Princeton University Press, Princeton, NJ).

Brainard, M.S. & Doupe, A.J. (2002). What songbirds teach us about learning. *Nature, 417*, 351–358.

Breiman, L., Friedman, J., Stone, C.J., & Olshen, R.A. (1984). Classification and Regression Trees (Chapman and Hall/CRC, Florida).

Brenowitz, E.A. & Beecher MD (2005). Song learning in birds: diversity and plasticity, opportunities and challenges. *Trends in Neuroscience, 28*, 127-132.

Brown, S.D. & Bottjer, S.W. (1993). Testosterone-induced changes in adult canary brain are reversible. *Journal of Neurobiology, 24*, 627-640.

Byers, B.E. & King, D.I. (2000). Singing by female chestnut-sided warblers. *Wilson Bulletin, 112*, 547-550.

Byers, B.E. & Kroodsma, D.E. (1992). Development of two song categories by chestnut-sided warblers. *Animal Behaviour, 44*, 799–810.

Carouso-Peck, S. & Goldstein, M. H (2018). Linking vocal learning to social reward in the brain: Proposed mechanisms of socially guided song learning. In *Oxford Handbook of Voice Perception*, Sascha Ühholz and Pascal Belin, Eds. Oxford University Press.

Carouso-Peck, S. & Goldstein, M.H. (2019a). Female social feedback reveals non-imitative mechanisms of vocal learning in zebra finches. *Current Biology, 29(4)*, 631–636.

Carouso-Peck, S. & Goldstein, M. H. (2019b). "A Bird's-Eye View of Communication". Scientific American, March 29.

Carouso-Peck, S., Menyhart, O., DeVoogd, T.J., & Goldstein, M.H. (submitted). Contingent parental responses guide zebra finch song learning in naturalistic social conditions.

Catchpole, C.K. & Baptista, L.F. (1988). A test of the competition hypothesis of vocal mimicry, using song sparrow imitation of white-crowned sparrow song. *Behaviour, 106*, 119–128.

Chaiken, M., Böhner, J. & Marler, P. (1993). Song acquisition in European starlings, *Sturnus vulgaris*: a comparison of the songs of live-tutored, tape-tutored, untutored, and wild-caught males. *Animal Behaviour, 46*, 1079–1090.

Chaiken, M., Böhner, J. & Marler, P. (1994). Repertoire turnover and the timing of song acquisition in European starlings. *Behaviour, 128*, 25–39.

Chaiken, M., Gentner, T.Q. & Hulse, S.H. (1997). Effects of social interaction on the development of startling song and the perception of these effects by conspecifics. *Journal of Comparative Psychology, 111*, 379–392.

Chen, Y., Clark, O., & Woolley, S.C. (2017). Courtship song preferences in female zebra finches are shaped by developmental auditory experience. *Proceedings of the Royal Society B, 284*, 20170054.

Chow, C.P., Mitchell, J.F., & Miller, C.T. (2015). Vocal turn-taking in a non-human primate is learned during ontogeny. *Proceedings of the Royal Society B, 282*, 20150069.

Cimprich, D. & Moore, F. (1995). Gray Catbird (*Dumetella carolinensis*). *The Birds of North America No. 167*., eds Poole, A. & Gill, F. (The Academy of Natural Sciences, Washington, D.C. and The American Ornithologists' Union, Philadelphia, PA).

Clarke, T., Orgill, C., & Dudley, T. (2006). *Field Guide to the Birds of the Atlantic Islands* (Christopher Helm, London, UK).

Crance, J.L., Bowles, A.E., & Garver, A. (2014). Evidence for vocal learning in juvenile male killer whales, *Orcinus orca*, from an adventitious cross-socializing experiment. *Journal of Experimental Biology*, *217,* 1229-1237.

Cunningham, M.A. & Baker, M.C. (1983). Vocal learning in white-crowned sparrows: sensitive phase and song dialects. *Behavioral Ecology and Sociobiology, 13*, 259–269.

Curtis, H.S. (1972). The Albert lyrebird in display. *Emu, 72*, 81-84.

Derrickson, K.C. (1987). Behavioral correlates of song types of the Northern Mockingbird (*Mimus polyglottos*). *Ethology, 74*, 21–32.

Derrickson, K. & Breitwisch, R. (1992). Northern mockingbird (*Mimus polyglottos*). *The Birds of North America, Vol. 7,* eds Poole, A., Stettenheim, P., Gill, F. (The Academy of Natural Sciences, Philadelphia, PA and The American Ornithologists Union, Washington, DC) pp 1-24.

Deviche, P. & Gulledge, C.C. (2000). Vocal control region sizes of an adult female songbird change seasonally in the absence of detectable circulating testosterone concentrations. *Journal of Neurobiology, 42*, 202-211.

Dittus, W.P.J. & Lemon, R.E. (1969). Effects of song tutoring and acoustic isolation on the song repertoires of cardinals. *Animal Behaviour, 17*, 523–533.

Dolbeer, R.A. (1978). Movement and migration patterns of red-winged blackbirds: A continental overview. *Bird-Banding, 49*, 17-34.

Dolby, A.S., Clarkson, C.E., Haas, E.T., Miller, J.K., Havens, L.E., & Cox, B.K. (2005). Do song-phrase production rate and song versatility honestly communicate male parental quality in the Gray Catbird? *Journal of Field Ornithology, 76*, 287–292.

Eales, L.A. (1989). The influences of visual and vocal interaction on song learning in zebra finches. *Animal Behaviour, 37*, 507-508.

Eisrer, L. (1976). *The American Robin: A Backyard Institution* (Taylor Trade Publications, Chicago).

Emmenegger, T., Hahn, S., & Bauer, S. (2014). Individual migration timing of common nightingales is tuned with vegetation and prey phenology at breeding sites. *BMC Ecology, 14*, 9.

Eriksen, A., Lampe, H.M., & Slagsvold, T. (2009). Interspecific cross-fostering affects song acquisition but not mate choice in pied flycatchers, *Ficedula hypoleuca*. *Animal Behaviour, 78*, 857–863.

Espmark, Y.O. & Lampe, H.M. (1993). Variations in the song of the pied flycatcher within and between breeding seasons. *Bioacoustics, 5*, 33–65.

Ficken, M.S. & Ficken, R.W. (1965). Comparative ethology of the chestnut-sided warbler, yellow warbler, and American redstart. *Wilson Bulletin, 77*, 363-375.

Freeberg, T.M., West, M.J., King, A.P., Duncan, S.D., & Sengelaub, D.R. (2002). Cultures, genes, and neurons in the development of song and singing in brown-headed cowbirds (*Molothrus ater*). *Journal of Comparative Physiology A, 188*, 993–1002.

Gayk, Z.G. & Lindsay, A.R. (2012). Winter microhabitat foraging preferences of sympatric boreal and black-capped chickadees in Michigan's upper peninsula. *Wilson Journal of Ornithology, 124*, 820-824.

Gentner, T.Q., Fenn, K.M., Margoliash, D., & Nusbaum, H.C. (2006). Recursive syntactic pattern learning by songbirds. *Nature, 440*, 1204-1207.

Ghazanfar, A.A., Liao, D.A., & Takahashi, D.Y. (2019). Volition and learning in primate vocal behavior. *Animal Behaviour, 151,* 239-247.

Goldstein, M.H., King, A.P., & West, M.J. (2003). Social interaction shapes babbling: Testing parallels between birdsong and speech. *Proceedings of the National Academy of Sciences, 100 (13),* 8030 – 8035.

Goodson, J.L., Wilson, L.C., & Schrock, S.A. (2012). To flock or fight: neurochemical signatures of divergent life histories in sparrows. *Proceedings of the National Academy of Sciences, 109*, 10685-10692.

Grava, T., *Fairhurst, G.D., Avey, M.T., Grava, A., Bradley, J., Avis, J.L., Bortolotti, G.R., Sturdy, C.B., & Otter, K.A.* (2013). Habitat quality affects early physiology and subsequent neuromotor development of juvenile black-capped chickadees. *PLOS ONE, 8*, e71852.

Greenberg, R. (1984). *The Winter Exploitation Systems of Bay-Breasted and Chestnut-Sided Warblers in Panama* (University of California Press, Berkeley).

Greenberg, R., Olsen, B.J., & Etterson, M.A. (2010). Patterns of seasonal abundance and social segregation in inland and coastal plain swamp sparrows in a Delaware tidal marsh. *Condor, 112*, 159-167.

Gros-Louis, J.G., West, M.J., Goldstein, M.H., & King, A.P. (2006). Mothers provide differential feedback to infants' prelinguistic sounds. *International Journal of Behavioral Development, 30 (6),* 509 – 516.

Hahn, A.H., Krysler, A., & Sturdy, C.B. (2013). Female song in black-capped chiackadees (*Poecile atricapillus*): acoustic song features that contain individual identity information and sex differences. *Behavioural Processes, 98*, 98-105.

Hailman, J.P. & Ficken, M.S. (1986). Combinatorial animal communication with computable syntax: chick-a-dee calling qualifies as 'language' by structural linguists. *Animal Behaviour, 34*, 1899–1901.

Halkin, S.L. & Linville, S.U. (1999). Northern Cardinal (*Cardinalis cardinalis*). *The Birds of North America, Vol. 440,* eds Poole, A., Gill, F. (The Birds of North America Inc., Pennsylvania, PA) pp 1-32.

Hamilton, K.S., King, A.P., Sengelaub, D.R., & West, M.J. (1998). Visual and song

nuclei correlate with courtship skills in brown-headed cowbirds. *Animal Behaviour, 56*, 973-982.

Harcus, J.L. (1973). Song studies in the breeding biology of the Catbird, *Dumetella carolinesis* (Aves: Mimidae) (thesis, University of Toronto, Canada).

Harrap, S. (1996). *Tits, Nuthatches and Treecreepers* (Christopher Helm Publishers, London).

Healy, K., McNally, L., Ruxton, G.D., Cooper, N., & Jackson, A.L. (2013). Metabolic rate and body size are linked with perception of temporal information. *Animal Behaviour, 86*, 685-696.

Herkert, J.R., Kroodsma, D.E. & Gibbs, J.P. (2001). Sedge Wren (*Cistothorus platensis). The Birds of North America, No. 582,* eds Poole, A., Gill, F. (The Birds of North America, Inc., Philadelphia, PA).

Higgins, P.J., Peter, J.M., & Steele, W.K. (2001). *Handbook of Australian, New Zealand and Antarctic Birds. Volume 5: Tyrant-flycatchers to Chats*. (Oxford University Press, Melbourne, Victoria).

Honda, E. & Okanoya, K. (1999). Acoustical and syntactical comparisons between songs of the white-backed munia *Lonchura striata* and its domesticated strain, the Bengalese finch *Lonchura striata* var. *domestica*. *Zoological Science, 16,* 319–326.

Houx, B.B. & ten Cate, C. (1998). Do contingencies with tutor behaviour influence song learning in zebra finches? *Behaviour, 135*(5), 599-614.

Howard, R.D. (1974). The influence of sexual selection and interspecific competition on mockingbird song (*Mimus polyglottos*). *Evolution, 28,* 428–438.

Hultsch, H. & Todt, D. (1989). Song acquisition and acquisition constraints in the nightingale, Luscinia megarhynchos. *Naturwissenschaften, 76,* 83–85.

Immelmann, K. (1969). Song development in the zebra finch and other estrildid finches. *Bird Vocalizations,* ed Hinde, R.A. (Cambridge: Cambridge University Press) pp 61-81.

Jahn, A.E., Bravo, S.P., Cueto, V.R., Levey, D.J., & Morales, M.V. (2012). Patterns of partial avian migration in northern and southern temperate latitudes of the New World. *Emu, 112,* 17-22.

Jäntti, A., Hakkarainen, H., Kuitunen, M., & Suhonen, J. (2007). The importance of

landscape structure for nest defense in the Eurasian Treecreeper *Certhia familiaris*. *Ornis Fennica, 84*, 145–154.

Jarvis, E.D. (2007). Neural systems for vocal learning in birds and humans: a synopsis. *Journal of Ornithology, 148*, S35–S44.

Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K., & Mooers, A.O. (2012). The global diversity of birds in space and time. *Nature, 491*, 444-448.

Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K., Redding, D., & Mooers, A.O. (2014). Distribution and conservation of global evolutionary distinctness in birds. *Current Biology, 24*, 1-12.

Johnson, S.L. (2006). Do American robins acquire songs by both imitating and inventing? *Wilson Journal of Ornithology, 118*, 341–352.

Johnson, D.M., Stewart, G.L., Corley, M., Ghrist, R., Hagner, J., Ketterer, A., McDonnell, B., Newsom, W., Owen, E., & Samuels, P. (1980). Brown-headed cowbird (*Molothrus ater*) mortality in an urban winter roost. *Auk, 97*, 299-320.

Jones, A. & Slater, P. (1996). The role of aggression in song tutor choice in the zebra finch: Cause or effect? *Behaviour, 133*, 103-115.

Kennerley, P. & Pearson, P. (2010). *Reed and Bush Warblers.* (Christopher Helm Publishers, London, UK).

Kessel, B. (1953). Distribution and migration of the European starling in North America. *Condor, 55*, 49-67.

Keys, G.C. & Dugatkin, L.A. (1990). Flock size and position effects on vigilance, aggression, and prey capture in the European starling. *Condor, 92*, 151-159.

Kiefer, S., Sommer, C., Scharff, C., Kipper, S., & Mundry, R. (2009). Tuning towards tomorrow? Common nightingales *Luscinia megarhynchos* change and increase their song repertoires from the first to the second breeding season. *Journal of Avian Biology, 40*, 231–236.

Kiefer, S., Sommer, C., Scharff, C., & Kipper, S. (2010). Singing the popular songs? Nightingales share more song types with their breeding population in their second season than in their first. *Ethology, 116*, 619–626.

King, A.P. & West, M.J. (1983). Epigenesis of cowbird song – a joint endeavor of males and females. *Nature, 305*, 704-706.

King, A.P., Freeberg, T.M., & West, M.J. (1996). Social experience affects the process and outcome of vocal ontogeny in two populations of cowbirds (*Molothrus ater*). *Journal of Comparative Psychology, 110*, 276–285.

Kirn, J.R., Clower, R.P., Kroodsma, D.E., & DeVoogd, T.J. (1989). Song-related brain regions in the red-winged blackbird are affected by sex and season but not repertoire size. *Journal of Neurobiology, 20*, 139-63.

Kirwan, G.M. & Green, G. (2012). *Cotingas and Manakins*. Princeton University Press.

Konishi, M. (1965). The role of auditory feedback in the control of vocalization in the white-crowned sparrow. *Zeitschrift für Tierpsychologie, 22*, 770–783.

Kroodsma, D.E. (1984). Songs of the Alder Flycatcher (*Empidonax alnorum*) and Willow Flycatcher (*Empidonax traillii*) are innate. *Auk, 101*, 13–24.

Kroodsma, D.E. (1986). Song development by castrated marsh wrens. *Animal Behaviour, 34*, 1572–1575.

Kroodsma, D.E. & Verner, J. (1978). Complex singing behaviors among Cistothorus wrens. *Auk, 95*,703–716.

Kroodsma, D.E. & Verner, J. (1987). Use of song repertoires among marsh wren populations. *Auk, 104*, 63–72.

Kroodsma, D.E., Meservey, W.R., & Pickert, R. (1983). Vocal learning in the Parulinae. *Wilson Bulletin, 95*, 138–140.

Kroodsma, D.E., Albano, D.J., Houlihan, P.W., & Wells, J.A. (1995). Song development by black-capped chickadees (*Parus atricapillus*) and Carolina chickadees (*P. carolinensis*). *Auk, 112*, 29–43.

Kroodsma, D.E., Bereson, R.C., Byers, B.E., & Minear, E. (1989). Use of song types by the Chestnut-sided Warbler: evidence for both intra- and inter-sexual functions. *Canadian Journal of Zoology, 67*, 447–456.

Kroodsma, D.E., Houlihan, P.W., Fallon, P.A., & Wells, J.A. (1997). Song development by grey catbirds. *Animal Behaviour, 54*, 457–464.

Kroodsma, D.E., Liu, W-C., Goodwin, E., & Bedell, P.A. (1999). The ecology of song improvisation as illustrated by North American sedge wrens. *Auk, 116*, 373–386.

Kroodsma, D.E., Sánchez, J., Stemple, D.W., Goodwin, E., Da silva, M.L., & Vielliard, J.M. (1999). Sedentary life style of Neotropical sedge wrens promotes song imitation. *Animal Behaviour, 57*, 855-863.

Kroodsma, D., Hamilton, D., Sánchez, J.E., Byers, B.E., Fandiño-Mariño, H., Stemple, D.W., Trainer, J.M., Powell, G.V.N. (2013). Behavioral evidence for song learning in the suboscine bellbirds (Procnias spp.; Cotingidae). *Wilson Journal of Ornithology, 125*, 1–14.

Laskey, A.R. (1936). Fall and winter behavior of mockingbirds. *Wilson Bulletin, 48*, 241-255.

Lehrman, D.S. (1970). Semantic and conceptual issues in the nature-nurture problem. In: *Development and Evolution of Behavior: Essays in Memory of T. C. Schneirla.* Eds. L. R. Aronson., E. Tobach, D. S. Lehrman & J. S. Rosenblatt. San Francisco: W. H. Freeman, pp. 1 7-52.

Leighton, G.M. (2017). Cooperative breeding influences the number and type of vocalizations in avian lineages. *Proceedings of the Royal Society B, 284*, 20171508.

Leitner, S. & Catchpole, C.K. (2007). Song and brain development in canaries raised under different conditions of acoustic and social isolation over two years. *Developmental Neurobiology, 67*, 1478–1487.

Leitner, S., Nicholson, J., Leisler, B., DeVoogd, T.J., & Catchpole, C.K. (2002). Song and the song control pathway in the brain can develop independently of exposure to song in the sedge warbler. *Proceedings of the Royal Society B, 269*, 2519–2524.

Lemon, R.E. & Scott, D.M. (1966). On the development of song in young cardinals. *Canadian Journal of Zoology, 44*, 191–197.

Letunic, I. & Bork, P. (2016). Interactive Tree Of Life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research, 44*, W242-5.

Lindsay, W.R., Houck, J.T., Giuliano, C.E., & Day, L.B. (2015). Acrobatic courtship display coevolves with brain size in manakins (Pipridae). *Brain, Behavior, and Evolution, 85*, 29-36.

Lovette, I.J., Arbogast, B.S., Curry, R.L., Zink, R.M., Botero, C.A., Sullivan, J.P., Talaba, A.L., Harris, R.B., Rubenstein, D.R., Ricklefs, R.E., & Bermingham,

E. (2012). Phylogenetic relationship of the mockingbirds and thrashers (Aves: Mimidae). *Molecular Phylogenetics and Evolution, 63*, 219-229.

MacKay, B.K. (2001). *Bird Sounds: How and Why Birds Sing, Call, Chatter and Screech.* (Stackpole Books, Mechanicsburg, PA).

Marler, P. (1966). Comparative study of song development in emberizine finches. *Proceedings of the 1st International Ornithological Congress,* 231-244.

Marler, P. (1970). A comparative approach to vocal learning: Song development in white-crowned sparrows. *Journal of Comparative and Physiological Psychology, 71*, 1-25.

Marler, P., Kreith, M., & Tamura, M. (1962). Song development in hand-raised Oregon juncos. *Auk, 79,* 12–30.

Marler, P., Mundinger, P., Waser, M.S., & Lutjen, A. (1972). Effects of acoustical stimulation and deprivation on song development in red-winged blackbirds (*Agelaius phoeniceus*). *Animal Behaviour,* 20, 586–606.

Marler, P. & Peters, S. (1977). Selective vocal learning in a sparrow. *Science, 198,* 519–521.

Marler, P. & Peters, S. (1982). Structural changes in song ontogeny in the swamp sparrow *Melospiza georgiana*. *Auk, 99,* 446–458.

Marler, P. & Peters, S. (1987). A sensitive period for song acquisition in the song sparrow, Melospiza melodia: a case of age-limited learning. *Ethology, 76*, 89–100.

Marler, P. & Peters, S. (1988a). The role of song phonology and syntax in vocal learning preferences in the song sparrow, *Melospiza melodia*. *Ethology, 77*, 125–149.

Marler, P. & Peters, S. (1988b). Sensitive periods for song acquisition from tape recordings and live tutors in the swamp sparrow, *Melospiza georgiana*. *Ethology, 77*, 76–84.

Mathewson, H.A., Morrison, M.L., Loffland, H.L., & Brussard, P.F. (2012). Ecology of willow flycatchers (*Empidonax traillii*) in the Sierra Nevada, California: Effects of meadow characteristics and weather demographics. *Ornithological Monographs, 75*, 1-32.

Meanley, B. (1965). The roosting behavior of the red-winged black-bird in the

Southern United States. *Wilson Bulletin, 77*, 217-228.

Mooney, R. (1999). Sensitive periods and circuits for learned birdsong. *Current Opinion in Neurobiology, 9*, 121–127.

Mountjoy, J.D. & Lemon, R.E. (1995). Extended song learning in wild European starlings. *Animal Behaviour, 49*, 357–366.

Musser, W.B., Bowles, A.E., Grebner, D.M., & Crance, J.L. (2014). Differences in acoustic features of vocalizations produced by killer whales cross-socialized with bottlenose dolphins. *Journal of the Acoustical Society of America, 136*(4), 1990-2002.

Nelson, D.A. & Marler, P. (1994). Selection-based learning in bird song. *Proceedings of the National Academy of Science, 91*, 10498-10501.

Newton, I. (1967). The feeding ecology of the bullfinch (*Pyrrhula pyrrhula* L.) in southern England. *Journal of Animal Ecology, 36*, 721-744.

Newton, I. (2000). Movements of Bullfinches *Pyrrhula pyrrhula* within the breeding season. *Bird Study, 47*, 372–376.

Nicholson, J.S., Buchanan, K.L., Marshall, R.C., & Catchpole, C.K. (2007). Song sharing and repertoire size in the sedge warbler, *Acrocephalus schoenobaenus*: changes within and between years. *Animal Behaviour, 74*, 1585–1592.

Nicolai, J. (1959). Familientradition in der Gesangsentwicklung des Gimpels (*Pyrrhula pyrrhula L.). Journal of Ornithology, 100*, 39–46.

Nolan, V., Ketterson, E.D., Cristol, D.A., Rogers, C.M., Clotfelter, E.D., Titus, R.C., Schoech, S.J., & Snajdr, E. (2002). Dark-eyed Junco (*Junco hyemalis*). *The Birds of North America, Vol. 716*, eds Poole A, Gill F (The Birds of North America, Inc., Philadelphia, PA) pp 1-44.

Nordby, J.C., Campbell, S.E., & Beecher, M.D. (2002). Adult song sparrows do not alter their song repertoires. *Ethology, 108*, 39–50.

Nottebohm, F. (1966). The role of sensory feedback in the development of avian vocalizations (thesis, Univ. of California, Berkley).

Nottebohm, F. (1971). Neural lateralization of vocal control in a passerine bird. I. Song. *Journal of Experimental Zoology, 177*, 229–261.

Nottebohm, F. (1972). The origins of vocal learning. *American Naturalist, 106*, 116-

140.

Nottebohm, F. & Nottebohm, M.E. (1978). Relationship between song repertoire and age in the canary, *Serinus canarius*. *Zeitschrift für Tierpsychologie, 46,* 298–305.

Nottebohm, F., Nottebohm, M.E., & Crane, L. (1986). Developmental and seasonal changes in canary song and their relation to changes in the anatomy of song-control nuclei. *Behavioral Neural Biology, 46,* 445–471.

Nulty, B., Burt, J.M., Akçay, Ç., Templeton, C.N., Campbell, S.E., & Beecher, M.D. (2010). Song learning in song sparrows: relative importance of autumn vs. spring tutoring. *Ethology, 116,* 653–661.

O'Loghlen, A.L. & Rothstein, S.I. (2002). Ecological effects on song learning: delayed development is widespread in wild populations of brown-headed cowbirds. *Animal Behaviour, 63,* 475–486.

Ota, N., Gahr, M., & Soma, M. (2015). Tap dancing birds: the multimodal mutual courtship display of males and females in a socially monogamous songbird. *Scientific Reports, 5,* 16614.

Otter, K., McGregor, P.K., Terry, A.M.R., Burford, F.R.L., Peake, T.M., & Dabelsteen, T. (1999). Do female great tits (Parus major) assess males by eavesdropping? A field study using interactive song playback. *Proceedings of the Royal Society B, 266,* 1305–1309.

Ortega, C. (1998). *Cowbirds and Other Brood Parasites* (University of Arizona Press, Tucson).

Payne, R.B. (1981). Song learning and social interaction in indigo buntings. *Animal Behaviour, 29,* 688–697.

Payne, R.B. (1991). Natal dispersal and population structure in a migratory songbird, the indigo bunting. *Evolution, 45,* 49-62.

Pavlova, D., Pinxten, R., Eens, M. (2005). Female song in European starlings: sex differences, complexity, and composition. *Condor, 107,* 559-569.

Payne, R.B., Payne, L.L., & Doehlert, S.M. (1984). Interspecific song learning in a wild chestnut-sided warbler. *Wilson Bulletin, 96,* 292–294.

Peabody, P.B. (1927). On the Alder Flycatcher (*Empidonax trailli alnorum*). *Wilson Bulletin, 39,* 20-23.

Poirier, C., Henry, L., Forasté-Mathelier, M., Lumineau, S., Cousillas, H., & Hausberger, M. (2004). Direct social contacts override auditory information in the song-learning process in starlings (*Sturnus vulgaris*). *Journal of Comparative Psychology, 118*, 179–193.

Poulsen, H. (1959). Song learning in the Domestic Canary. *Zeitschrift für Tierpsychologie, 16*, 173–178.

Powell, G.V. & Bjork, R.D. (2003). Habitat linkagaes and the conservation of tropical biodiversity as indicated by seasonal migrations of three-wattled bellbirds. *Conservation Biology, 18*, 500-509.

Price, P.H. (1979). Developmental determinants of structure in zebra finch song. *Journal of Comparative and Physiolgical Psychology, 93*, 260–277.

Reichard, D.G., Brothers, D.E., George, S.E., Atwell, J.W., & Ketterson, E.D. (2017). Female Dark-eyed Juncos (Junco hyemalis thurberi) produce male-like song in a territorial context during the early breeding season. *Avian Biology, 49*, 1-6.

Rice, J.O. & Thompson, W.L. (1968). Song development in the indigo bunting. *Animal Behaviour, 16*, 462–469.

Riebel, K. & Slater, P.J.B. (1998). Male chaffinches (*Fringilla coelebs*) can copy calls from a tape tutor. *Journal of Ornithology, 139*, 353–355.

Ritchison, G. (1986). The singing behavior of female northern cardinals. *Condor, 88*, 156-159.

Robinson, F.N. & Curtis, H.S. (1996). The vocal displays of the lyrebirds (Menuridae). *Emu, 96*, 258–275.

Rogers, C.M., Theimer, T.L., Nolan, V., & Ketterson, E.D. (1989). Does dominance determine how far dark-eyed juncos, *Junco hyemalis*, migrate into their winter range? *Animal Behaviour, 37*, 498-506.

Sedgwick, J. (2004). Site fidelity, territory fidelity, and natal philopatry in Willow Flycatchers (Empidonax traillii). *Auk, 121*, 1103-1121.

Shackleton, S.A. & Ratcliffe, L. (1933). Development of song in hand-reared black-capped chickadees. *Wilson Bulletin, 105*, 637-644.

Sibley, C.G. & Ahlquist, J.E. (1990). Phylogeny and Classification of Birds (Yale University Press, New Haven, Conn.).

Sirkiä, P.M. & Laaksonen, T. (2009). Distinguishing between male and territory quality: females choose multiple traits in the pied flycatcher. *Animal Behaviour, 78*, 1051–1060.

Skjerven, J. (2011). Learning of song in the Pied Flycatcher (*Ficedula hypoleuca*) – a field study (thesis, University of Oslo, Norway).

Slater, P.J.B. & Ince, S.A. (1982). Song development in chaffinches: what is learnt and when? *Ibis, 124*, 21–26.

Slowtow, R. & Rothstein, S.I. (1995). Influence of social status, distance from cover, and group size on feeding and vigilance in white-crowned sparrows. *Auk, 112*, 1024-1031.

Smith, V.A., King, A.P., & West, M.J. (2000). A role of her own: female cowbirds, *Molothrus ater*, influence the development and outcome of song learning. *Animal Behaviour, 60*, 599-609.

Snow, B.K. (1977). Territorial behavior and courtship of the male three-wattled bellbird. *Auk, 94*, 623-645.

Snow, D.W. (1982). The Cotingas: Bellbirds, Umbrella birds and their allies. (British Museum Press, London, UK).

Snowdon, C.T., Elowson, A.M., & Roush, R.S. (1997). Social influences on vocal development in New World primates. In *Social Influences on Vocal Development*, eds. Snowdon, C.T. & Hausberger, M. (Cambridge University Press, Cambridge, UK).

Sober, S.J. & Brainard, M.S. (2009). Adult birdsong is actively maintained by error correction. *Nature Neuroscience, 12*, 927–931.

Soha, J.A. & Marler, P. (2000). A species-specific acoustic cue for selective song learning in the white-crowned sparrow. *Animal Behaviour, 60(3)*, 297-306.

Soha, J.A. & Marler, P. (2001a). Cues for early discrimination of conspecific song in the white-crowned sparrow (*Zonotrichia leucophrys*). *Ethology, 107*, 813–826.

Soha, J.A. & Marler, P. (2001b). Vocal syntax development in the white-crowned sparrow (*Zonotrichia leucophrys*). *Journal of Comparative Psychology, 115*, 172-180.

Sossinka, R. & Böhner, J. (1980). Song types in the zebra finch *Poephila guttata castanotis*. *Zeitschrift für Tierpsychologie, 53*, 123–132.

Summers, D.D.B. (1979). Bullfinch dispersal and migration in relation to fruit bud damage. *British Birds, 72*, 249-263.

Swann, R.L. (1988). Are all large chaffinch flocks composed of continentals? *Ringing & Migration, 9*, 1-4.

Takahashi, D.Y., Liao, D.A., & Ghazanfar, A.A. (2017). Vocal learning via social reinforcement by infant marmoset monkeys. *Current Biology, 27,* 1844-52.

Therneau, T.M. & Atkinson, B. (2009). Package: rpart. Available at http://cran.r-project.org/web/packages/rpart/rpart.pdf.

Thielcke, G. (1977). Die Programmierung von Vogelgesängen. *Vogelwarte, 29*, 153–159.

Thielcke, G. (1984). Gesangslernen beim Gartenbaumläufer (*Certhia brachydactyla*). *Vogelwarte, 32*, 282–297.

Thielcke, G. (1986). Constant proportions of mixed singers in tree creeper populations (*Certhia familiaris). Ethology, 72*, 154–164.

Thielcke, G. & Krome, M. (1991). Chaffinches *Fringilla coelebs* do not learn song during autumn and early winter. *Bioacoustics, 3*, 207–212.

Thielcke, G. & Wustenberg, K. (1985). Experiments on the origin of dialects in the short-toed tree creeper (*Certhia brachydactyla*). *Behavioral Ecology and Sociobiology, 16,* 195-201.

Tietze, D.T., Martens, J., Sun, Y-H., & Päckert, M. (2008). Evolutionary history of treecreeper vocalisations (Aves: Certhia). *Organisms Diversity & Evolution, 8*, 305–324.

Thompson, W.L. (1970). Song variation in a population of indigo buntings. *Auk, 87*, 58–71.

Thompson, W.L. (1972). Singing behavior of the indigo bunting, *Passerina cyanea*. *Zeitschrift für Tierpsychologie, 31*, 39–59.

Thorpe, W.H. (1958). The learning of song patterns by birds with especial reference to the song of the chaffinch *Fringilla aiclchs*. *Ibis, 100,* 535-70.

Thorpe, W.H. (1961). *Bird Song* (Cambridge University Press, London).

Titus, R.C., Ketterson, E.D., & Nolan, V. (1997). High testosterone prior to song crystallization inhibits singing behavior in captive yearling dark-eyed juncos

(*Junco hyemalis*). *Hormones and Behavior, 32*, 133–140.

Todd, P.M. & Miner, O.F. (1993). Parental guidance suggested: how parental imprinting evolves through sexual selection as an adaptive learning mechanism. *Adaptive Behavior, 2*, 5-47.

Todt, D., Hultsch, H., & Heike, D. (1979). Conditions affecting song acquisition in nightingales (Luscinia megarhynchos L.). *Zeitschrift für Tierpsychologie, 51*, 23–35.

Verner, J. (1965). Time budget of the male long-billed marsh wren during the breeding season. *Condor, 67*, 125-139.

Verner, J. (1975). Complex song repertoire of male long-billed marsh wrens in eastern Washington. *Living Bird, 14*, 263-300.

Waser, M.S. & Marler, P. (1977). Song learning in canaries. *Journal of Comparative and Physiological Psychology, 91*, 1–7.

Wauer, R.H. (1999). *The American Robin*. (University of Texas Press, Austin, Texas).

Welter, W.A. (1935). The natural history of the long-billed marsh wren. *Wilson Bulletin, 47*, 3-34.

West, M.J. & King, A.P. (1985). Learning by performing: An ecological theme for the study of vocal learning. *Issues in the Ecological Study of Learning*, eds Johnston, T.D. & Piertrewicz, A.T. (Lawrence Erlbaum Associates).

West, M.J. & King, A.P. (1988). Female displays affect the development of male song in the cowbird. *Nature, 334*, 244-246.

West, M.J., Stroud, A.N., & King, A.P. (1983). Mimicry of the human voice by European starlings: the role of social interaction. *Wilson Bulletin, 95*, 635–640.

Wilkinson, R. (1990). Vocal behavior and call development in the bullfinch (*Pyrrhula pyrrhula). Bioacoustics, 2*, 179-197.

Williams, H. (2004). Birdsong and singing behavior. *Annals of the New York Academy of Science, 1016*, 1-30.

Williams, H., Kilander, K., & Sotanski, M.L. (1993). Untutored song, reproductive success and song learning. *Animal Behaviour, 45*, 695−705.

Wingfield, J.C. (1994). Regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. *Hormones and Behavior, 28*, 1-15.

Wolfenbarger, L.L. (1998). Red coloration of male northern cardinals correlates with mate quality and territory quality. *Behavioral Ecology, 10*, 80-90.

Wooley, S.M.N. & Rubel, E.W. (1997). Bengalese finches *Lonchura striata domestica* depend upon auditory feedback for the maintenance of adult song. *Journal of Neuroscience, 17*, 6380-6390.

Yamaguchi, A. (2001). Sex differences in vocal learning in birds. *Nature, 411*, 257–258.

Yasukawa, K., Blank, J.L., & Patterson, C.B. (1980). Song repertoires and sexual selection in the red-winged blackbird. *Behavioral Ecology and Sociobiology, 7*, 233–238.

Zann, R.A. (1996). *The Zebra Finch: A Synthesis of Field and Laboratory Studies* (Oxford University Press, Oxford).